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Starch formation in the leucoplasts of Phaseolus vulgaris

PAULINE YOUNG

(WITH PLATE 1)

It is generally assumed that, in the transformation of soluble carbohydrate to insoluble starch in the plastid, intermediate products are formed. In the absence of definite knowledge of the chemical constitution of such compounds it is quite commonly assumed that we are dealing here with a reversible reaction and that clues to the "Aufbau" of starch will be found in the products of its hydrolysis.

Mikosch (1885) called attention to a zone surrounding the young starch grain in the leucoplast which he believed was the "mother substance" from which new starch was formed. Salter (1898) also described such an area, which he interprets as a zone of starch of greater density than that of rest of the grain.

Denniston (1905) observed a distinct orange-staining layer around growing starch grains of *Canna* stained in Flemming's triple stain. He regards this layer as being composed of a transitional material. He observes that a new layer of starch first appears as a narrow violet-stained crescent in the midst of the orange zone. This isolated violet zone gradually widens in the direction of the latest formed layer of starch until the enclosed part of the orange zone is completely replaced with violet-staining starch. The latest part of the layer to be deposited is pale violet as contrasted with the deep violet first-formed part of the lamella. Denniston suggests that the formation of the new layer may be due to the condensation of carbohydrate material from smaller carbohydrate-like molecules, brought about by the abstraction of water, or other similar chemical change. The suggestion is also made that the orange zone is already a carbohydrate material which is later transformed into starch. The latter suggestion is regarded as being the most probable.

Although not attempting to explain the cause of stratification, Denniston regards the phenomenon as being due to the layers of varying densities in the starch grain. Those layers retaining the most stain are regarded as the most dense on the assumption that the stain is removed with less difficulty from the less dense layers.

The material for this study was fixed in weak chrom-acetic mixture, in Benda's fixative and in Merkel's fixative, the time being from 24 to 48 hours. The cotyledons of the young seeds were used in all cases. When very small, the entire cotyledon was fixed, after dissecting it out of the

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seed coat. Larger cotyledons were cut into small pieces before fixation. In the case of the very youngest cotyledons, portions of the entire pod were fixed. Portions of living cotyledons were also used with vital stains and with the iodine test and Fehling's reaction.

Most of the sections were stained with Flemming's triple stain. With this stain the nucleoli and certain bodies, probably protein in nature, stain red; the body of the plastids stains deep orange, the cytoplasm a distinctly paler orange and the starch grains violet. Not all the structures that stain violet are necessarily starch, but any substances within the plastid retaining the violet stain is quite obviously starch. This has been proved conclusively by tests made with iodine and with the polariscope. With Heidenhain's iron haematoxylin, with the fixatives used, the cytoplasm stains gray, the plastids blackish and the starch remains unstained.

The so-called vital stains, methylene blue, neutral red and Janus green, were used in an attempt to determine the nature and the origin of the leucoplasts. Other sections were treated with a modified Millon's solution (the Sachs test for dextrin) with the hope of obtaining some light on the composition of the vacuolate centers of the smaller plastids.

In the living cells of very young cotyledons there are minute spherical bodies in the cytoplasm adjacent to the cell membrane. With neutral red solution (1–500) the bodies stain a dark red in contrast with the paler red of the cytoplasm. In these same cells slightly larger bodies have the appearance of being hollow, showing a dark red peripheral region and light central areas, with the neutral red. This appearance is probably to be interpreted as showing a dense protoplasmic shell surrounding either a watery protoplasmic center or a non-protoplasmic vacuole. With Janus green and methylene blue the smaller bodies stain respectively green and blue, and the larger ones show respectively green and blue borders with light centers.

When tested with iodine, the lighter vacuole-like centers of the bodies in question show no positive color reaction. The iodine test also gives negative results with cells which have been treated with a chloral hydrate solution in order to swell any starch inclusions in the central area. Examination of this central region with the polariscope shows no figures characteristic of starch. Thus the above results indicate that there is no starch in these central regions at this stage in the development of these bodies.

With Fehling's solution the lighter central areas show a light brick red color which disappears upon being placed in absolute alcohol. The old Sachs test for dextrin was a brick red color reaction with Fehling's solution which was not removed with absolute alcohol. This test has been

discredited (Zimmerman, 1893) since pure dextrin cannot reduce Fehling's solution.

A brick red color with Fehling's solution, even though not permanent, is very significant and indicates that an incipient reduction of the copper has taken place due, no doubt, to the presence of some aldehyde carbohydrate.

In the fixed and sectioned material of these youngest cotyledons, the cytoplasm of the peripheral layer contains minute rounded bodies which lie right at the limit of clear vision, probably $\frac{1}{4}$ micron or less. These are stained orange in Flemming's triple stain (fig. 2a). These orange-staining bodies occupy the same position in the cell, are similar in size to those bodies which stain red with neutral red in unfixed material. They are undoubtedly identical with them. Other bodies slightly larger, $\frac{1}{2}$ micron in diameter, in fixed material, show minutely granular, orange-stained peripheral layers with paler central regions that contain no starch and show no traces of granularity (fig. 2b). These are comparable in size and general appearance to the bodies showing thick dark red borders and light centers when stained with neutral red in fresh material. This pale orange central area is the same as the central region which reacts light brick red with Fehling's solution in the slightly larger bodies of the fresh cells.

The cells of the fresh cotyledons a few days older than those referred to above contain, in addition to the previously discussed rounded bodies, others noticeably larger. These bodies when stained with neutral red show dark red peripheries and have the lighter centers somewhat larger than those already described. These appear homogeneous throughout, but when tested with iodine there is to be seen in its central part a definitely marked area which gives the characteristic reaction for starch. Around this minute blue-stained starch grain there is a zone of the central area which shows no reaction for starch. These larger bodies with small starch grains in their lighter central areas are very similar to those smaller ones, the central areas of which show no starch. The central portion of the larger bodies is, however, often eccentric so that the orange-staining peripheral regions are thicker at one end.

In fixed material of the same age, these earliest starch grains retain none of the violet of Flemming's triple stain (fig. 3a). With the polariscope they show the figures characteristic of starch. With Flemming's triple stain considerable care must be taken to keep the violet dye in the large grains even, so it is not surprising that in these very smallest grains the stain is lost.

It seems clear that the deep orange-staining zones surrounding the

small starch grains are to be regarded as leucoplasts. These are similar to and occupy the same position with relation to the central areas as those of the smaller bodies with central zones containing no starch. It may therefore be concluded that the latter are also plastids. These can be traced back through an unbroken series to those small bodies which lack the lighter central zone and which are therefore also plastids or primordia of plastids (fig. 4b). These smaller plastids are very minute and numerous in the young cells and it is impossible to make any definite statement as to their origin. In no case, however, were they observed to be dividing, so that the assumption that they arise *de novo* from the cytoplasm seems justified.

In sections from older cotyledons, stained with Flemming's triple stain, the stages in the development of the starch grain may be observed. As has already been made clear, there appears in the smaller, deep orange-staining plastids, a central area of a texture and staining reaction different from the periphery (fig. 5a) which shows no indication of starch with the polariscope. Slightly larger plastids (fig. 5b–g) show very minute crosses with the polariscope; they do not occupy the whole of the central area. In the centers of still larger plastids these small starch grains now stain readily with the crystal violet and they do not fill entirely this central area but are surrounded by a zone staining light orange (fig. 5h). The starch grain thus develops in the midst of this material which gives the temporary reaction with Fehling's solution.

In the formation of a second layer of starch around this first minute grain there is deposited around it a paler violet-staining layer (fig. 5i). The orange-staining layer between the plastid and the starch grain tends to maintain its original thickness although, according to Denniston's observations on *Canna*, it is very probable that a part of it is utilized in the formation of the new layer. In a few cases, in the larger starch grains, a dark layer surrounds a paler center (fig. 6a). It may be that these are transverse sections of elongated starch grains cut near the end, since none of the smaller ones were seen with light central layers surrounded by a darker layer.

The growing starch grain (fig. 6c) is composed of distinct, alternating layers of dark and light violet-staining material with a light orange-staining layer, similar to that designated by Denniston (1905) as the transitional zone lying between the starch grain and the plastid material. There is no gradual transition from the light staining starch to the darker staining in each lamella as in the starch grain of *Canna* and other similar starch grains. The number of layers is not as great as in the *Canna* or potato starch grain, usually not exceeding five or six.

In the bean starch grain new layers are formed at the outer extremity

of the latest formed old layer of the starch grain. In case the latest formed layer stains pale violet, there arises at its outer extremity a layer staining distinctly darker (fig. 6b–c). This layer widens gradually and when it has grown to the average thickness there is formed around it another layer staining pale violet. Thus the new layers are growing into the orange-staining zone from the latest formed layer of starch. It seems obvious that with actively growing starch grains there is an area within the plastid which is not starch but which has a chemical composition intermediate between the soluble carbohydrates and starch.

With the older starch grains the deep orange-staining plastids which surround them are often thicker at one end so as to form a cap-like structure (fig. 6b–c). In such starch grains the developing layer of starch arises earliest on that end of the grain where the cap-like part of the leucoplast is found and gradually grows around the grain forming a layer approximately uniform in thickness.

Some of the starch grains which are apparently not growing do not have the light orange zone so that the starch is in direct contact with the granular plastid (fig. 6d). It seems reasonable to assume that these plastids are temporarily inactive.

DISCUSSION

It cannot be said that the question of the origin of plastids in the vascular plants has ever been fully settled. Schimper's view of the continuity of the plastid through the life cycle of the plant, while somewhat discredited in the vascular plants, has not been disproved beyond all possibility of doubt. On the other hand the origin of the plastid *de novo*, from undifferentiated cytoplasm has not been finally proved. Recently Miss Stone (1932) has presented evidence of the presence of chlorophyll in undifferentiated cytoplasm of cells of young leaves of potato and condensation of this chlorophyll-bearing cytoplasm to form chloroplasts is described. It seems surprising that such obvious condensations of cytoplasm to form chloroplasts as Miss Stone describes could have been overlooked so long. Her account of this phenomenon will undoubtedly stimulate new work in this phase of plastid formation. More recently Kiyohara (1935) has described plastids in the eggs and pollen tubes of certain angiosperms and is of the opinion that his findings present positive proof of Schimper's view.

In *Phaseolus vulgaris* undoubted leucoplasts containing very small starch grains may be traced through an unbroken series to minute structures which are obviously also plastids. Since these are very small and very numerous it is impossible to determine their origin, but there is no evi-

dence of their having arisen from similar bodies by division. Although the exact origin has not been determined, the most reasonable conclusion seems to be that they have arisen *de novo* from the cytoplasm. It seems very probable that plastids may be formed in a different manner in the different plant groups and in different phases in the life cycle of the same group. For example, multiplication by fission is easily followed throughout the period of growth of many algae, while in the higher plants it is difficult to find stages of plastid fission except in fully developed chloroplasts.

As has already been observed by Mikosch (1885) and Salter (1898), the very young plastid becomes differentiated into a peripheral layer and a central vacuole-like layer previous to the beginning of starch formation. In *Phaseolus* the peripheral layer is noticeably granular and stains quite strongly with orange G. The layer is regarded as the active or protoplasmic part of the leucoplast. The central region is homogeneous and lacking in granularity and, in *Phaseolus*, stains faintly with orange G. Although this central area differs from the peripheral layer in texture and refractivity, as well as staining reaction, it is not impossible that it is protoplasmic and may be regarded as a part of the leucoplast which either is being transformed or is engaged in transforming some carbohydrate into starch.

The color reaction of the central area of the leucoplast with Fehling's solution is of considerable significance and points to the presence of an appreciable amount of an aldehyde carbohydrate. That the reaction is not permanent may indicate that glucose or other actively reducing carbohydrate is not present. The appearance of the young starch grain, following so closely upon the appearance of this vacuolar stage, suggests very strongly that polysaccharide molecules of considerable size are present in this central area.

The difference between the deposition of starch in *Phaseolus vulgaris* and in *Canna* is of considerable interest. In the former, new layers are apparently formed by a transformation of that part of the orange-staining zone adjacent to the latest formed old layer of the starch grain and growth is away from the center. On the other hand, according to Denniston (1905), new layers in the *Canna* starch grain appear first as crescent-shaped masses in the midst of the thick part of the eccentric orange-staining zone, and gradually extended toward the last formed layer until it is reached. The layers in the starch grains in *Phaseolus* stain alternatingly dark and light with iodine or gentian violet, with no gradual transition of the one into the other. In *Canna* there is a gradual transition in each layer from dark violet on the outside to light violet on the inside of the layer.

Starch grains of certain other plants, as some of the cereal grains, show no lamellae at all. Zwikker (1921) has attempted to demonstrate that the

presence or absence of lamellae is correlated with an excess or deficiency of certain electrolytes. Wheat starch, showing no distinct lamellae, normally contains an excess of calcium ions, while potato starch with its conspicuous lamellae contains an excess of potassium ions.

This work of Zwikker is but one of many on the chemistry of the starch grain from a colloid-chemical view point. From Samec's (1927) exhaustive review of the subject it will be seen that it is one of great complexity. No substantial agreement has been reached as to the relation of the carbohydrate starch to the mineral constituents of the starch grain. The data accumulated, however, point clearly to a relationship between such elements as phosphorus, silicon, potassium and calcium and the characteristic reaction of different starch grains to malt extract and other hydrolytic agents, the formation of colloidal pastes and probably the characteristics of their lamella formation. The difference in the lamellation in the starch grains of *Phaseolus* and *Canna* may very well be connected with characteristic differences in the mineral content of the grains.

The starch grain is clearly not a simple structure made up of the carbohydrate starch with the mineral constituents adhering in an indefinite manner to its exterior, but a close association exists between these two types of components. The mineral compounds may lie between the micellae which make up the composite starch crystal or may even be in loose chemical combination with them.

SUMMARY

1. Very young leucoplasts contain vacuole-like central areas which do not react as starch but which show a distinct brown-red color reaction with Fehling's solution, which color is removed by absolute alcohol. This reaction must indicate the presence of an aldehyde carbohydrate.

2. The young starch grains originate in the vacuole-like central areas of these plastids.

3. Surrounding the growing starch grain there is a persistent zone having similar color reactions to the vacuole-like area.

4. New layers of starch are formed at the outer extremity of the last formed old starch grain and grow outward.

This work was completed at the botanical laboratories of the University of Texas. The writer wishes to express her sincere appreciation to Professor Frederick McAllister for his assistance and criticisms.

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Explanation of plate 1

All drawings were made with the aid of a camera lucida the magnification being 1750 times.

Fig. 1. Portion of a cell from a very young cotyledon stained with Heidenhain's iron haematoxylin showing the minute plastids (a) and the slightly larger plastids (b) with vacuole-like centers containing no starch.

Fig. 2. Portion of a cell from a very young cotyledon stained in Flemming's triple stain showing minute orange-stained plastids (a) and slightly larger orange-stained plastids with vacuole-like centers containing no starch.

Fig. 3. Portion of a cell from a cotyledon a few days older, stained with Flemming's triple stain, showing in addition to the smaller plastids, those which contain small starch grains which have not retained the violet of the stain.

Fig. 4. Entire cell from an older cotyledon stained with Heidenhain's stain, showing various stages in the development of the plastids—the larger ones containing small starch grains.

Fig. 5. Successive stages in the development of young starch grains, from an old cotyledon, stained with Flemming's triple stain.

a. Small plastid with vacuole-like center containing no starch.

b–g. Plastids containing small starch grains that do not retain the violet stain.

h. Plastid showing violet-staining young starch grain surrounded by the light orange zone.

i. Plastid showing a young, dark violet starch grain with a pale violet layer surrounded by a pale orange zone.

Fig. 6. Entire cell from an old cotyledon showing starch grains in various stages of development. Stained with Flemming's stain.

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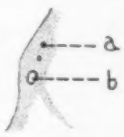

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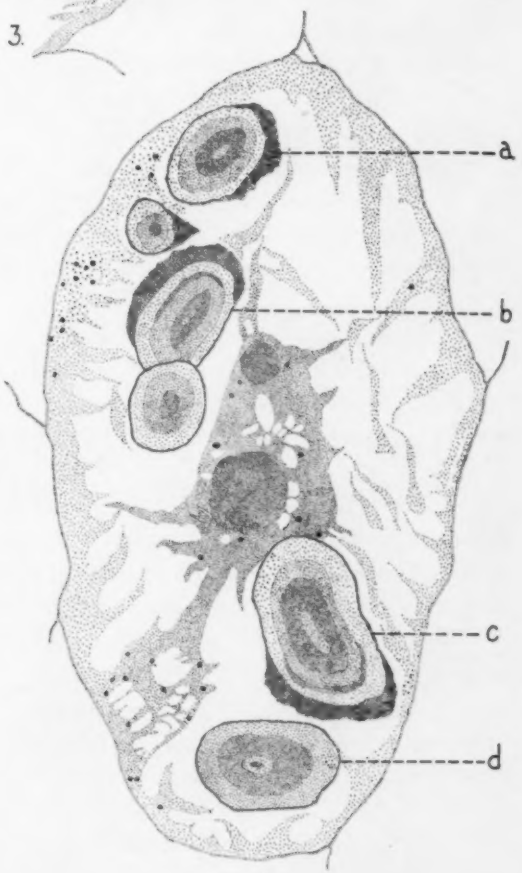

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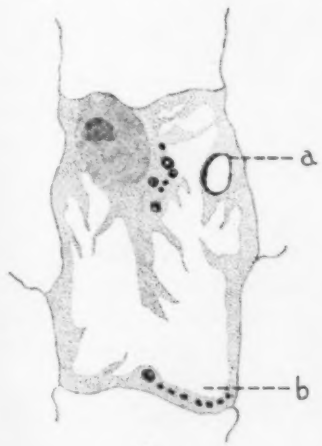

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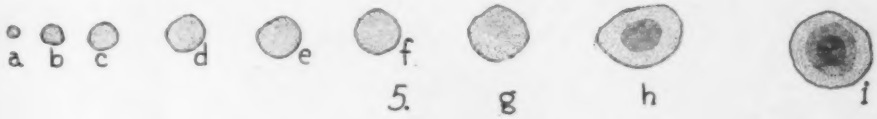

5.

YOUNG: LEUCOPLASTS

The development of the embryo of Zizania aquatica in the seed and in artificial culture

CARL D. LARUE AND GEORGE S. AVERY, JR.

(WITH EIGHT FIGURES)

The normal embryogeny of certain grasses is well known: *Avena fatua* L. (Cannon, 1900), *Poa annua* L. (Souèges, 1924) and *Zea mays* L. (Randolph, 1936). The embryos of these three species, upon their development into seedlings are representative of two of the three morphological types distinguished by Van Tieghem (1897). It has been shown since that they are all fundamentally one type, appearing differently upon development because of the difference in location of the meristematic region in the first internode (Avery, 1930). If the species indicated above may be considered typical of the family, the general course of embryogeny is the same for the approximately 5000 species (Bews, 1929) of grasses. In the structural aspects of its development into a seedling *Zizania* follows closely that of *Avena*.

Although the culture of plant embryos *in vitro* has been demonstrated many times (Hannig, 1904; Dietrich, 1924; Tukey, 1934; etc.), the culture of embryos starting with relatively young stages is of recent date; apparently no comparative studies have been made of embryogeny *in vivo* and *in vitro*.

It has been pointed out (LaRue, 1936) that in maize the cotyledon ceases to grow when the embryos are cultured on agar. The question at once arises as to whether embryos in culture continue to develop as "normal" embryos, or, whether their normal growth and form is dependent upon their presence within the tissues of the parent plant.

The embryo of *Zizania* was chosen for this study because it possesses (1) a cotyledon which changes in the course of its development from a flat circular disc to a markedly linear organ. In the course of this development its width increases only five times, but its length 125 times, i.e., in normal embryogeny it shows marked polarized growth. It possesses (2) an epiblast which undergoes changes similar to those of the cotyledon but lesser in degree. Such changes in the length-width ratio of these two structures are so pronounced that any departure from normal embryogeny may be detected.

Is the growth and form of the cotyledon (and epiblast) inherent in the genotype, or is its development subject to environmental circumstances?

MATERIALS AND METHODS

Embryos were removed from ovaries by dissection under a low-power

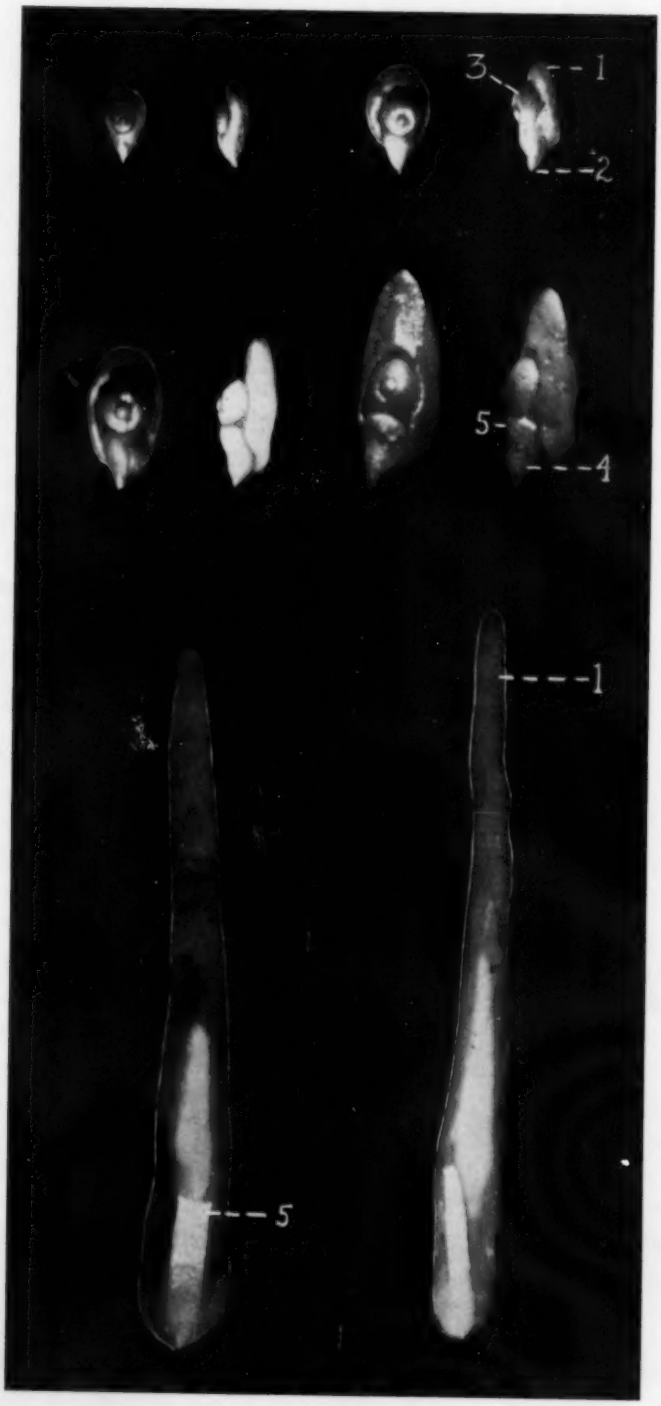

Fig. 1. (*See opposite page for legend*)

binocular microscope. They ranged in stage of development from the smallest which could be removed readily (0.2 mm. in diameter) to fully grown embryos. Aside from the sterilization of all dissecting instruments, etc., no further sterilization was attempted; little difficulty was encountered in removing the embryos aseptically, and placing them on agar media in Petri dishes.

The culture medium used was made up with the salts of White's (1934) solution, two per cent cane sugar and one per cent agar-agar. The yeast extract of White's solution which had been found unnecessary for the growth of maize embryos (LaRue, 1936), was omitted.

For studies on normal embryogeny the embryos were dissected from their ovaries at different stages of development, fixed in formalin acetic alcohol or Nawashin's solution, imbedded in paraffin, etc. The artificially cultured embryos were grown for 5 days in diffuse light or in the dark under ordinary laboratory conditions. They were then fixed and imbedded by the same procedure as that used for the normal embryos; all were cut at a thickness of 6–10 microns and stained with fast green.

The 5-day period for the growth of the embryos was chosen because in that interval of time mature embryos reached the stage at which the shoot burst through the coleoptile thus marking the beginning of their development as seedling plants. The smaller the embryo (upon transfer to agar culture) the longer the time required for it to reach this stage. All but the smallest embryos were grown ultimately to the stage of rupture of the coleoptile, but the significant changes in their development were initiated, at least, within the 5-day period.

OBSERVATIONS

Gross development of normal embryos and embryos in culture. Since our aim was a study of comparative embryogeny *in vivo* and *in vitro*, no attempt was made to start with embryos smaller than could be removed readily (0.2 mm.). A study of the early phases of embryogeny *in vitro* awaits improved techniques; early development seemed less important to this study because the early stages are concerned more with increase in number of cells than with differentiation. We chose to study the period of development in which the embryo acquires its most characteristic features.

In order to determine the relative ages of the embryos used in this study, their normal embryogeny (in the developing seed) was studied in relation to time (table 1).

Fig. 1. Embryos of *Zizania aquatica:* 0.2mm., 0.3mm., 0.7mm., 0.9mm., 5mm., and 12mm. (under varying magnifications). 1, cotyledon; 2, suspensor; 3, coleoptile; 4, coleorhiza; 5, epiblast.

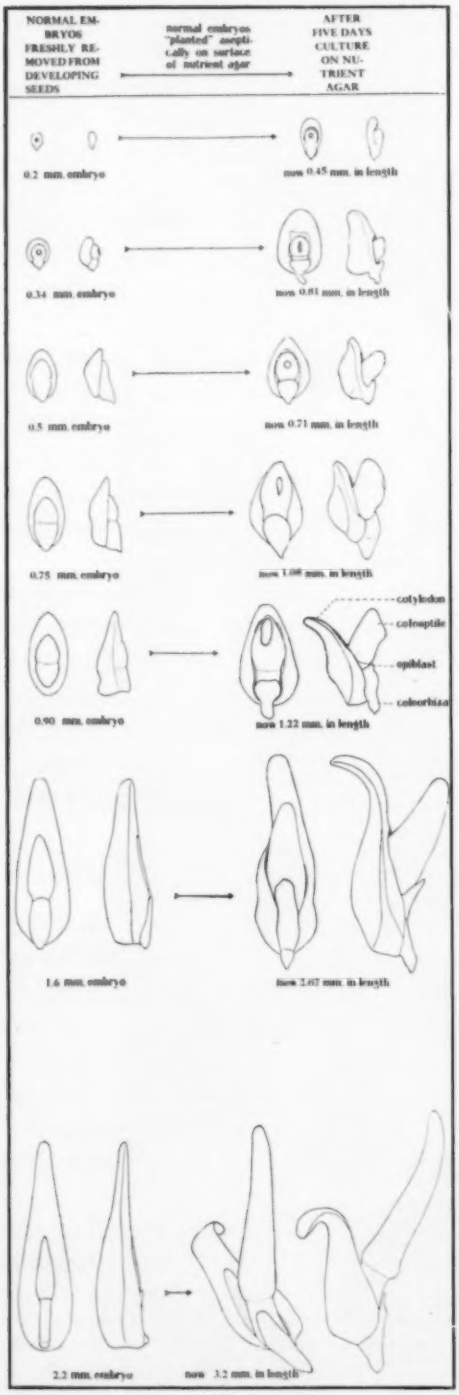
NORMAL EMBRYOS FRESHLY REMOVED FROM DEVELOPING SEEDS
normal embryos "planted" aseptically on surface of nutrient agar
AFTER FIVE DAYS CULTURE ON NUTRIENT AGAR
0.2 mm. embryo
now 0.45 mm. in length
0.34 mm. embryo
now 0.81 mm. in length
0.5 mm. embryo
now 0.71 mm. in length
0.75 mm. embryo
now 1.06 mm. in length
cotyledon
coleoptile
epiblast
coleorhiza
0.90 mm. embryo
now 1.22 mm. in length
1.6 mm. embryo
now 2.67 mm. in length
2.2 mm. embryo
now 3.2 mm. in length

TABLE 1

Data on growth in length of embryos of Zizania

DAYS AFTER POLLINATION	LENGTH OF EMBRYOS IN MM.
3	0.1
5	0.15
7	0.35
9	1.0
11	1.5
13	2.25
15	4.0
17	8.0
19	10.0

Upon removal to agar culture, the embryos of all but the smallest sizes grew readily. The mature (embryos 10–12 mm. in length) but "unripe" seeds required no rest period; when planted they would germinate at once. When once "ripened," however, the seeds would not germinate even if the pericarps were cut open, although the embryos if removed and placed on nutrient agar, grew satisfactorily.

A comparison of growth rate in normal embryogeny and cultured embryos is not possible. Total length-growth of an embryo in the seed is much greater than when in culture and presumably the increase in volume is of the same degree. An embryo

Fig. 2. *Zizania aquatica;* diagrams of normal embryos as removed from ovaries, and of similar embryos after 5 days culture on nutrient agar. Note the development of the coleoptile (and of the coleorhiza in the larger embryos) during the period of growth in culture.

2.2 mm. long (about 13 days after pollination) became 3.2 mm. long after 5 days in culture. If left undisturbed in the seed for this length of time, the same embryo would have increased to 8 mm. in length. But such a comparison obscures the facts; length-growth of the cultured embryos means growth of the coleoptile and first internode, while that of the normal embryo represents increase in length of the cotyledon.

The major differences between the two sets of embryos are differences in form. See figures 1 to 3 for illustrations of this development.

MICROSCOPIC OBSERVATIONS ON THE DEVELOPMENT OF THE NORMAL EMBRYOS AND EMBRYOS IN CULTURE

Embryos 0.2 to 0.35 mm. (figs. *1* and *2*) upon removal from ovaries often double their length after 5 days in culture, but undergo no further differentiation. The stem growing point, upper portion of the coleoptile, and the cotyledon are clearly dis-

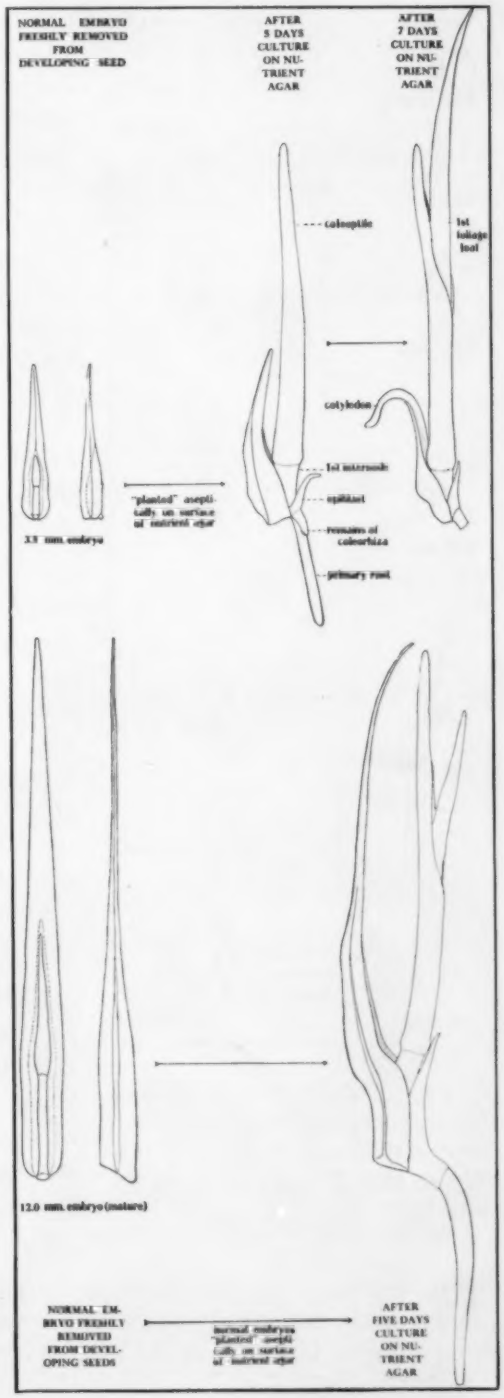
NORMAL EMBRYO FRESHLY REMOVED FROM DEVELOPING SEED
AFTER 5 DAYS CULTURE ON NUTRIENT AGAR
AFTER 7 DAYS CULTURE ON NUTRIENT AGAR
coleoptile
1st foliage leaf
cotyledon
1st internode
epiblast
remains of coleorhiza
primary root
3.2 mm. embryo
"planted" aseptically on surface of nutrient agar
12.0 mm. embryo (mature)
NORMAL EMBRYO FRESHLY REMOVED FROM DEVELOPING SEEDS
normal embryos "planted" aseptically on surface of nutrient agar
AFTER FIVE DAYS CULTURE ON NUTRIENT AGAR

Fig. 3. *Zizania aquatica;* continuation of fig. 2. The coleoptile, first internode, and primary root have elongated markedly after 5 days in culture; the cotyledon and the epiblast do not show further development.

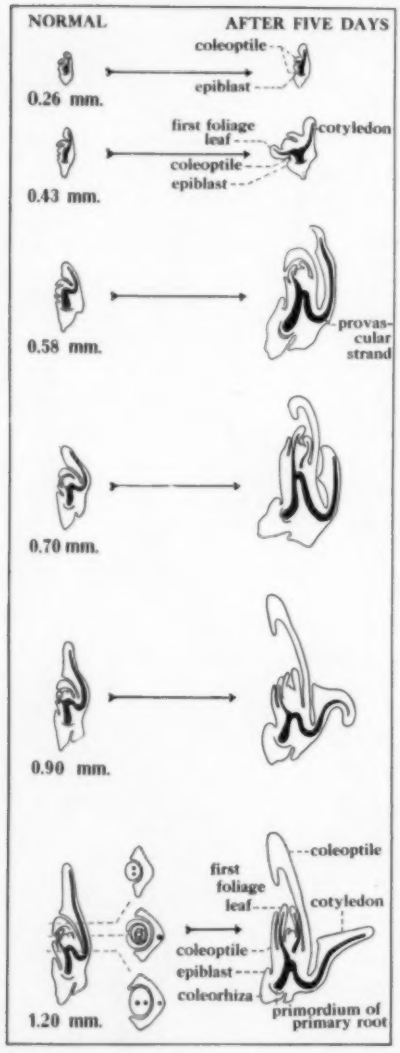

Fig. 4. *Zizania aquatica;* diagrams of median longisections of normal embryos of various sizes, and of similar embryos after 5 days culture on nutrient agar.

tinguishable (figs. *4* and *6*); the provascular strand is barely discernible in the latter. The root primordium, not yet differentiated in normal embryos of this size, fails to differentiate during the five days in culture.

Normal embryos 0.4 mm. or more in length at the time of transfer to agar, underwent the following structural changes in the course of five days in culture (figs. *4*, *5* and *7*): (1) The cotyledon and epiblast underwent a slight increase in size, but there was no evidence of cell division continuing in these structures after the transfer of the embryos to agar culture.

(2) The coleoptile in 0.4 to 0.7 mm. embryos increased in size as a result of the enlargement of its cells, but there was no evidence of a continuation of cell division; however, cell division in this structure continued in culture if the embryos were 0.9 mm. or longer at the time of transfer. The "pore" in the more or less conical coleoptile seldom closed in the embryos which were 1.5 mm. or less in length at the time of transfer to agar.

(3) Either the first foliage leaf or the first internode were the initial structures to undergo marked development when the embryos were transferred to agar; in either structure, there was a long period of cell division after the transfer.

(4) The primary root, although clearly differentiated, is the last structure to undergo discernible change after transfer of the embryos to culture. Its growth is slow in embryos less than about 3.0 mm. in length at the time of transfer.

DISCUSSION

It is clear that when embryos of *Zizania* were placed on nutrient agar, the cotyledon and epiblast ceased to grow; in contrast, the shoot primordium (and in older embryos, the primary root) departed from its slow growth and differentiation, and began what was virtually a precocious "germination" or development into the seedling stage (similar to the gross morphological development of maize embryos in culture, (LaRue, 1936). *Zizania* is an excellent subject for the study of comparative embryogeny *in vivo* and *in vitro* because the mature normal embryo possesses a cotyledon which extends the entire length of the fruit, often extending around the end of the caryopsis and downward on the back side; the epiblast also is unusually long and prominent. In embryos transferred to agar culture both these organs fail to continue their polarized growth. It is obvious that the continued elongation of these organs is a matter of embryonic development conditioned by factors present in the growing ovary. No investigation has been made to determine the nature of these factors, but whatever they may be, we know that normal embryogeny is absolutely dependent upon them. Removed

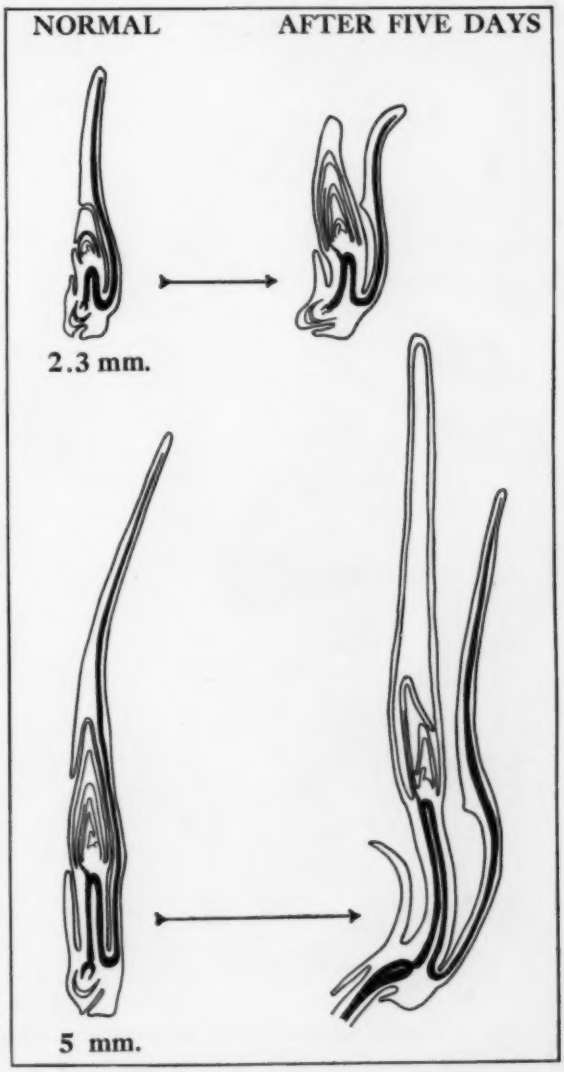

Fig. 5. *Zizania aquatica;* continuation of Fig. 4.

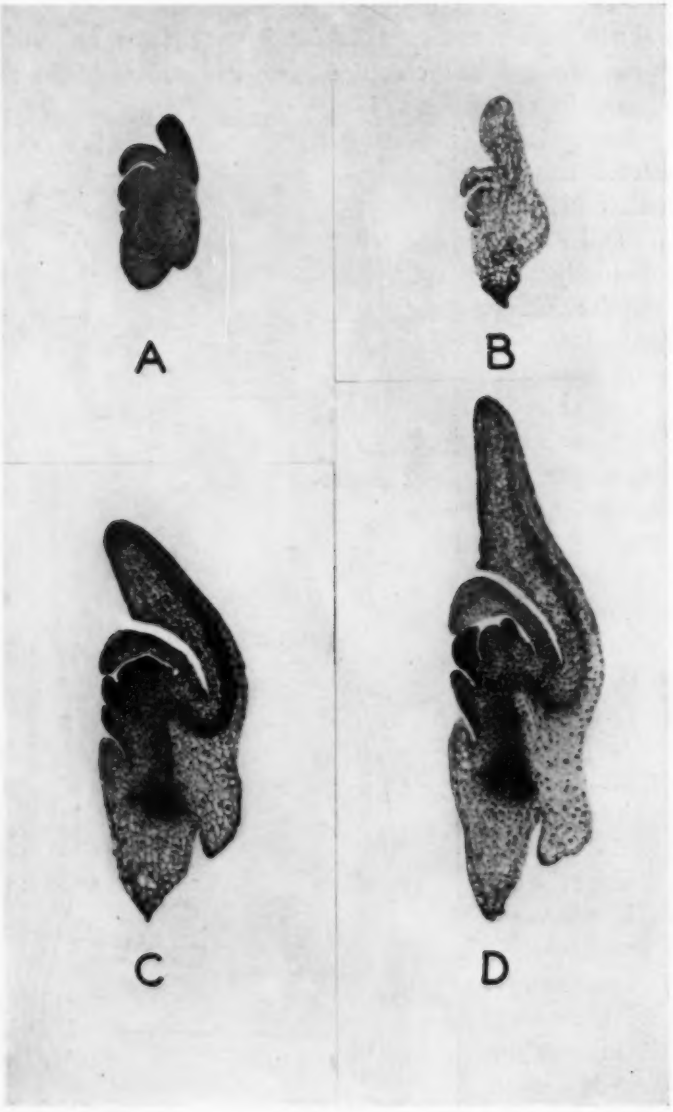

Fig. 6. *Zizania aquatica;* A, C, and D, photomicrographs of median longisections of normal embryos; A, 0.26 mm.; B, same, after 5 days in culture, now 0.43 mm.; C, 0.70 mm.; and D, 0.90 mm.

from their influence the embryo rapidly becomes a seedling, i.e., its axial development begins. The comparative study of embryos *in vivo* and *in vitro* offers a method of investigating the analysis of the factors which control normal embryogeny.

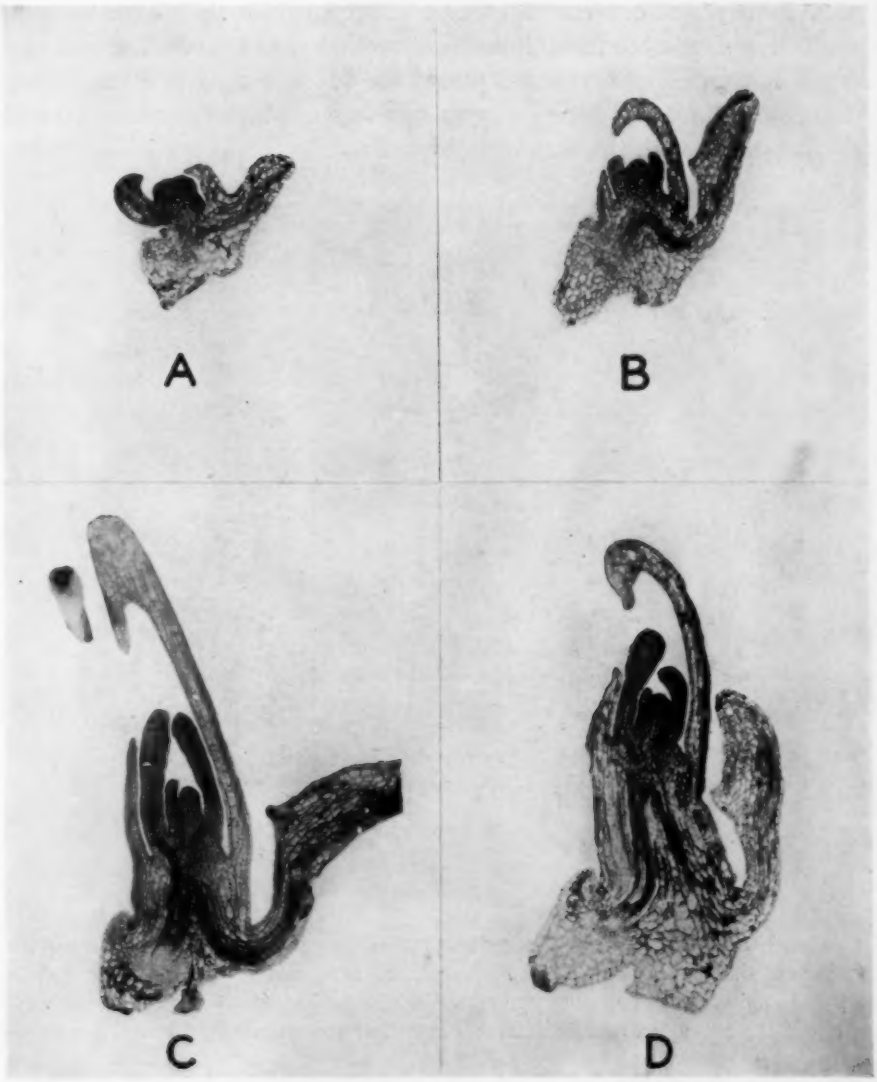

Fig. 7. *Zizania aquatica;* photomicrographs of median longisections of embryos of various sizes after 5 days culture on nutrient agar. Compare with fig. 4 for sizes of these embryos at time of removal from ovaries.

SUMMARY

1. The embryo of *Zizania aquatica* was chosen for this study because the marked elongation of the cotyledon and the epiblast during embryogeny *in vivo* provide a means of determining departures from the normal during development *in vitro*.

2. Embryos of different ages were dissected from their ovaries. Representative sizes were fixed, imbedded, sectioned and stained, and a corresponding set of embryos was placed on nutrient agar in Petri dishes. After 5 days in culture the embryos of this second group were sectioned for comparison with the normal embryos.

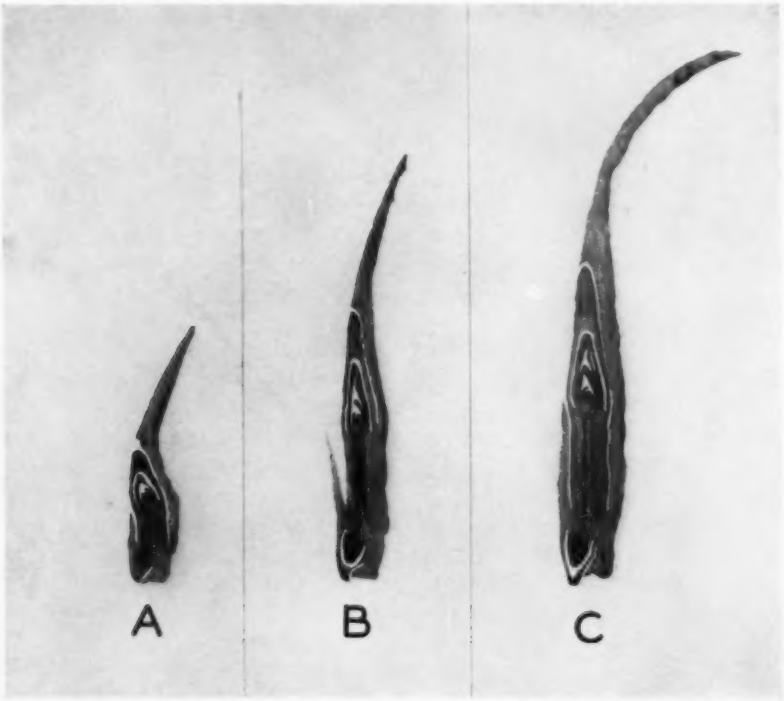

Fig. 8. *Zizania aquatica;* photomicrographs of longisections of normal embryos of different sizes. A, 2.3 mm.; B, 3.8 mm.; C, 5.2 mm. See fig. 5 for diagrams of A and C. Longisections of mature embryos differ in no important respect from C, except that the cotyledon usually attains a length of approximately 12 mm. See fig. 3.

3. Embryos of all but the smallest sizes were grown to the seedling stage in culture. Small embryos took a somewhat longer time to reach the seedling stage. Embryos smaller than 0.4 mm. failed to reach the seedling stage.

4. Determinations were made of the time required after pollination for the development of the different sizes of embryos, in the course of normal embryogeny.

5. The normal development of embryos has been traced and compared with that of those grown *in vitro*. Growth in culture is generally character-

ized by *precocious* development of the shoot and retarded growth of the primary root. In older embryos elongation of the primary root is not appreciably retarded. Growth of the cotyledon and epiblast is arrested upon transfer to agar culture. Normal growth and development of the embryo is dependent upon its presence within the tissues of the parent plant.

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[1] A part of this work was done while both authors were in residence at the University of Michigan Biological Station (no. 640 in the series of botanical papers from the Univ. of Mich.). All photomicrographs by Donald D. Irish, The Dow Chemical Company, Midland, Michigan.

A new green alga: Oedocladium Lewisii

L. A. WHITFORD

(WITH PLATE 2)

Although *Oedocladium protonema* has not been collected since the genus was established by Stahl, five additional species have been described. The writer was fortunate enough to collect a seventh in autumn, 1936. For several weeks it was widely distributed but not abundant in lower piedmont North Carolina; and in December it was collected in the extreme eastern part of the State. Like most of the species it is terrestrial and is always associated with *Vaucheria*, *Botrydium*, liverworts, and mosses. It forms tiny colonies on very moist sandy or loam soil, that was plowed the current year. Half shaded areas in bottom-land fields are the most likely collecting places. To the naked eye it resembles moss protonema but is slightly yellowish green and grows in scattered tufts one to two millimeters in diameter. Rainy or cloudy and cool weather for several weeks is probably necessary for it to become abundant enough to be readily collected.

Oedocladium Lewisii sp. nov.

Monoica; oogoniis singulis, plerumque terminalibus, globosis, operculo inferior. Oosporis globosis, membrana triplici, omne levi vel mesosporio angulato (in sectione optica undulato), oogonium complentibus. Cellulis suffulcio sine pigmentum, saepe moribundus. Antheridiis ad 9-cellularibus, gametis masculis singulis. Cellulis vegetativus subcylindricis, cellula terminali apice conica. Cellulis vegetativis superterraneis 13–22μ×60–106μ. cell. veg. subterraneis 4–11μ×ad 257μ. Oogonis 45–70μ diam. Oosporis 42–60μ. diam. Antheridiis 16–21μ latis, 12–21μ longis.

Herb. L. A. W. 550–554.

Monoecious; oogonium solitary, nearly always terminal, operculum inferior. Oospore globose with a triple membrane, the middle layer smooth or angulate (undulate in optical section) the other layers smooth, nearly or entirely filling the oogonium. Suffultory cell without color usually empty. Antheridia up to 9; sperm single. Vegetative cells subcylindric, terminal cells with conic apex, 13–22μ×60–106μ. Rhizoidal cells 4–11μ×(up to) 257μ. Oogonium 45–70μ×48–70μ. Oospore 42–60μ in diameter. Antheridium 16–21μ×12–21μ.

The species was collected in October and November at five stations in Wake County, North Carolina, and once each in Durham and Harnett counties. In late December it was also collected in Craven, Carteret, and Pamlico counties in the coastal plain.

There is a superficial resemblance to *O. Hazenii*, but the inferior operculum and empty suffultory cells readily separate it from all known species.

It is the second described species with an operculum and the other character seems to be unique for the genus. In the last division of the suffultory cell, all of the chlorophyll and most of the cytoplasm migrates into the forming oogonium. A nucleus and a small amount of much vacuolated cytoplasm remains in the suffultory cell. By the time the oospore is mature this protoplasm has usually disintegrated leaving a dead cell supporting the oogonium.

Observation of living, dividing cells and studies of preserved material by simple microchemical methods, lead the writer to believe that cell division occurs according to the theory of Kraskovitz for *Oedogonium*. That is, when the ring forms in a dividing cell, it forms not as an isolated mass of new cell wall material laid down at that particular point, but is merely an inward bulging portion of a new wall layer laid down, and completely covering the inside of the mother cell (figs. 6, 8). The mother cell ruptures under the inward bulging portion (ring) and this stretches or extends itself to form the wall of the daughter cell. The cross wall is not a "floating" structure but forms *in situ* at the point of attachment, and after the nucleus and cytoplasm have migrated into the daughter cell. Branches are formed by the failure of the mother cell to rupture all the way around. In this case the ring bulges out through the slit formed on the side of the mother cell and forms the first cell of the new branch. Aberrant L-shaped cells sometimes observed, are good proof that branches form in this way (fig. 7). Rhizoidal or semi-rhizoidal cells continue to grow by intussusception near the tip of the cell. In this case most of the protoplasm, and chlorophyll if present, is concentrated in the distal end, leaving the other end of the cell colorless and vacuolate.

A more complete description of cell formation in *Oedocladium* will be published later

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Explanation of plate 2

All drawings made with the aid of a camera lucida. Cells in figures 6, 7, and 8 swollen with 60–65% sulfuric acid.

Fig. 1. Young plant showing growth habit.

Fig. 2. Oogonium formation showing migration of chloroplast into the oogonium.

Fig. 3. Oogonium with colorless suffultory cell.

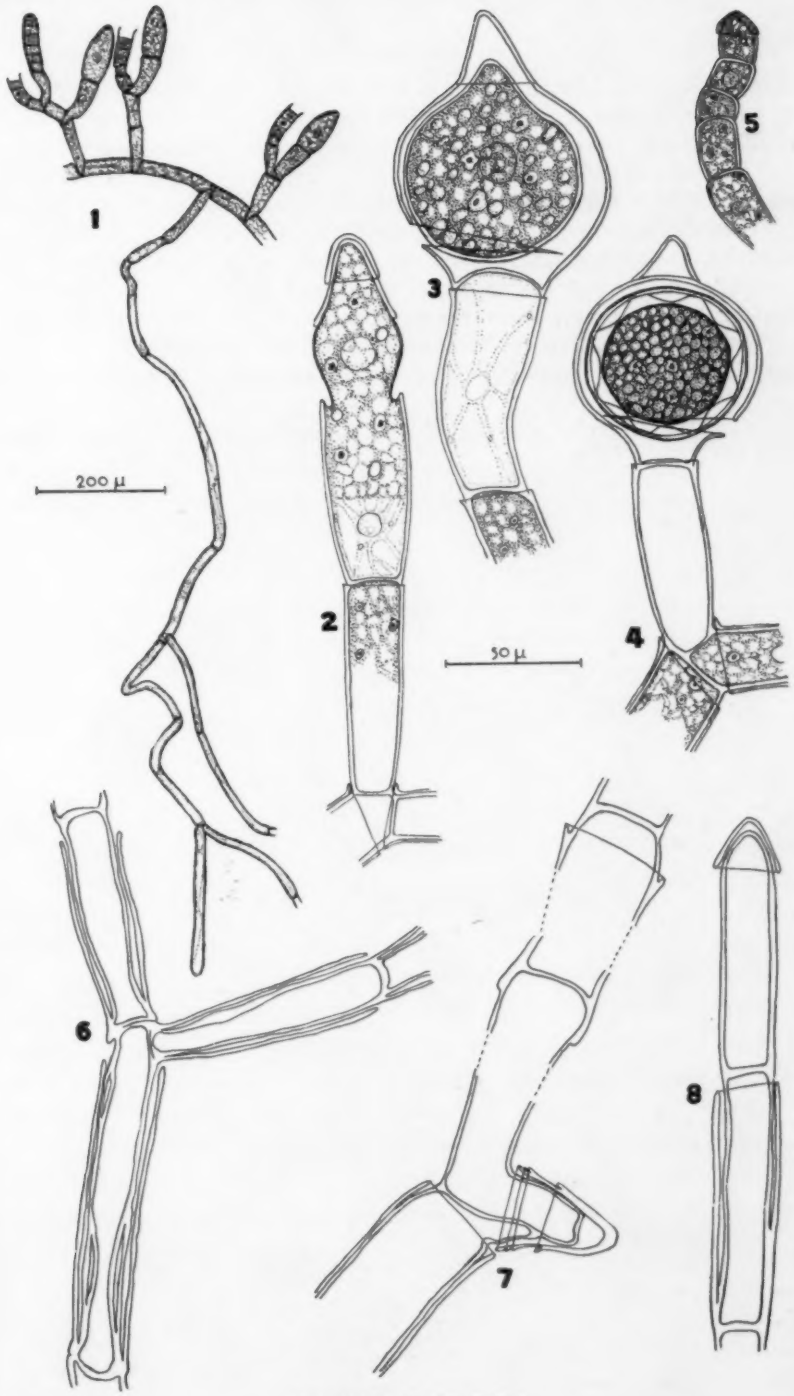
Fig. 4. Oogonium with mature oospore and empty suffultory cell.

Fig. 5. Antheridia.

Fig. 6. Cells swollen with strong sulfuric acid showing that after cell division has occurred cell walls have two or more complete layers.

Fig. 7. L-shaped cell resulting from incomplete rupture of mother cell wall.

Fig. 8. Showing that cell wall consists of one layer before division and of two layers after a cell has divided.



WHITFORD: OEDOCLADIUM

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Delphinium carolinianum and its allies

ROBERT F. MARTIN

Few genera of North American plants are more puzzling than the delphiniums. Among the entities differences in flower color, leaf segmentation, pubescence, and habit are often unaccompanied by tangible structural changes, and determination depends upon the weighing of several evasive and independently varying characters. The roots and seeds are reliable enough but they are, in general, essentially alike among closely allied species. Furthermore, a great deal of herbarium material lacks these organs.

No group is responsible for more confusion than the *Delphinium carolinianum* complex. The present notes result from examining a series of sheets preparatory to attempting a monograph of the genus in North America.

The first recognizable species of the group was described by Walter[1] as *D. carolinianum*. Michaux[2] redescribed this species as *D. azureum*, a name commonly encountered in literature and on herbarium labels. Dr. Blake[3] has pointed out that Walter's specimen is only a fragment and not determinable. I believe, however, it is safe to assume on the basis of distribution that Walter collected the species that has been accredited to him. More species have been added from time to time by Nuttall, D. Don, Huth, Greene, and Rydberg. Lately Miss Perry[4] has contributed a variety. Most confusion exists in the application of Huth's, Greene's, and Rydberg's names.

In my opinion, use of the orientation of the spur, its degree of curvature, and the distance of the bracteoles from the receptacle to distinguish entities is unjustified. Throughout the genus the upper part of the pedicel and the receptacle are bent in anthesis so that the flower is horizontal or turned downward. Later by unequal growth in the pedicel, it is moved through 90° or 180° to an erect position, and this same growth causes the bracteoles to become remote.

Unless otherwise indicated, all specimens cited are in the United States National Herbarium. Those in the herbarium of the National Arboretum are followed by N. A. Those specimens found in both collections are followed by U. S., N. A.

[1] Walter, T., Flora Caroliniana p. 155. 1788.
[2] Michaux, A., Fl. Bor. Amer. **1**: 314. 1803.
[3] Blake, S. F., Rhodora **17**: 130. 1915.
[4] Perry, L. M., Rhodora **39**: 20–22. 1937.

a. Seeds wing-margined
Pubescence glandular, at least in part. 1. *D. carolinianum* (typical).
Pubescence not glandular. 1a. *D. carolinianum*, var. *crispum*.
aa. Seeds squamellate, not wing-margined. b.
b. Flowers white, greenish or yellowish white, or blue tinged.
Pubescence glandular, at least in part. 2. *D. virescens* (typical).
Pubescence not glandular. 2b. *D. virescens* var. *camporum*.
bb. Flowers blue. 2a. *D. virescens*, var. *vimineum*.

1. D. CAROLINIANUM Walt. Fl. Car. 155. 1788. *D. azureum* Michx., Fl. Bor. Am. **1:** 314. 1803; *D. Nortonianum* Mack. & Bush, Trans. Acad. Sci. St. Louis **12:** 82. 1902; *D. azureum*, var. *Nortonianum* Palmer & Steyermark, Ann. Mo. Bot. Gard. **22:** 543. 1935; *D. carolinianum*, var. *Nortonianum* Perry, Rhodora **39:** 20. 1937.

I am unable to distinguish *D. Nortonianum* from large, deep blue states of *D. carolinianum*. In all its characters, size and color excepted, it seems identical.

Georgia to Texas and Missouri. Also from Florida to Virginia, according to Small.

GEORGIA: Bibb Co., Biltmore Herb (3767c); ALABAMA: Lee Co., *Pollard & Maxon 15;* MISSISSIPPI: Hinds Co., *Cook, May 24, 1925;* TEXAS: Walker Co., *Dixon 555;* Woods Co., *Reverchon 4341;* OKLAHOMA: Leflore Co., *Blakeley 1419;* ARKANSAS: Nevada Co., *Hollister 12;* MISSOURI: Jackson Co., *Bush 377*, isotype of *D. Nortonianum*.

1a. D. CAROLINIANUM, var. CRISPUM Perry, Rhodora **39:** 21. 1937.

In addition to the absence of glandular hairs, the carpels seem longer than in typical *D. carolinianum*. The specimen of Patterson here cited has mature seeds quite like those of the species.

Alabama to Arkansas, north to Missouri and Illinois.

ALABAMA: Perry Co., *Smith, 187(6?)*; ARKANSAS: Carroll Co., *Palmer 5585;* MISSOURI: Barry Co., *Bush 228;* ILLINOIS: Henderson Co., *Patterson*, no date given.

2. D. VIRESCENS Nutt. Gen. N. Am. Pl. **2:** 14. 1818: *D. azureum β virescens* Huth, Helios **10:** 34. 1892: *D. Penardi* Huth, loc. cit: *D. albescens* Rydb, Bull. Torrey Cl. **26:** 583. 1899: *D. carolinianum*, var. *Penhardii* A. Nels. in Coult. & Nels. New Man. Rocky Mtn. Bot. 193. 1909: *D. virescens*, var. *Penardi* Perry, Rhodora **39:** 21. 1937.

This is the common species of the prairies and plains, and ranges into the foothills of the Rockies. Huth's *D. Penardi*, I am unable to distinguish. Attempts have been made to separate it as the form with the upper part of the stem eglandular. It appears, however, that the glandular hairs may be present on any or all parts of the stem indiscriminately.

Louisiana and Texas, north to Colorado and Wisconsin.

LOUISIANA: Jeff Davis Parish, *Palmer 7672;* TEXAS: Brazos Co., *Palmer 9369;* Victoria Co., *Tracy 9243;* OKLAHOMA: Woods Co., *Stevens 551;* KANSAS: Riley Co., *Norton* (Kans. St. Ag. Col. 8); NEBRASKA: Grant Co., *Marsh, June 25, 1916,* (N. A., U. S.); SOUTH DAKOTA: Moody Co., *Ball 875;* MINNESOTA: Hennepin Co., *Mearns 270;* IOWA: Harrison Co., *Eggleston 15135;* COLORADO: Arapahoe Co., *Eggleston 11216,* (N. A., U. S.)

2a. D. **virescens,** var. **vimineum** (D. Don), comb. nov.: *D. vimineum* D. Don in Sweet Brit. Flow. Gard. Ser. 2, pl. 374. 1838: *D. azureum,* var. *vimineum* A. Gray, Bot. Gaz. **12:** 52. 1887: *D. macroseratilis* Rydb. Bull. Torrey Club **26:** 585. 1899.

Because of its rugose-squamellate seeds this form belongs here rather than with *D. carolinianum,* which it resembles in habit.

TEXAS: Harris Co., *Palmer 9620;* Kerr Co., *Heller 1723;*

2b. D. **virescens,** var. **camporum** (Greene), comb. nov.: *D. camporum* Greene Erythea **2:** 183. 1894: *D. Wootoni* Rydb., Bull. Torrey Club **26:** 587. 1899.

Doctor Greene's name has been considered a synonym of *D. Penardi* Huth and specimens of this variety are generally determined as *D. Penardi* It is clear, however, from Green's description and from plants determined by him that he described the eglandular plant of the Mexican border states. Huth's plant from his description was glandular and therefore, as I see it, the same as typical *D. virescens.* Var. *camporum* may be distinguished by the subscapose stem and often by the blue tinged flowers.

Texas to Arizona.

TEXAS: Bexar Co., *Havard, March 1882;* Terrell Co., *Palmer 33546;* NEW MEXICO: Dona Ana Co., *Wooton, May 26, 1905;* ARIZONA: Cochise Co., *Eggleston 19863.*

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The cultural characteristics of the species of Fomes[1,2]

W. A. Campbell

Within the past several years, the identification of the causal organisms of timber decay has become of considerable importance especially in connection with improvement cuttings in second-growth hardwood timber. Some investigators have been puzzled by the multiplicity of fungous forms which may be isolated from decayed wood and have felt that the positive identification of these fungi is a hopeless task. This study has been undertaken in order to work out a practical system whereby fungi from decayed wood may be identified and to study the cultural characteristics of the species of a single genus. The genus *Fomes* was selected because a number of isolates of the different species were already available and, since the sporophores are perennial, additional isolates could be obtained at any time.

REVIEW OF THE LITERATURE

Since Hartig's discovery of the relation between the mycelium of a fungus present in wood and the condition of decay which existed, many investigators have demonstrated that this mycelium, when isolated under aseptic conditions, could be made to grow upon artificial media in test tube and Petri dish cultures.

The following investigators have pointed out that cultural characteristics are valuable means for the identification of the organisms of decay: White (1910) first cultured a member of the genus *Fomes*, using *Fomes applanatus*, and made observations on cultural characteristics; Baxter (1924) pointed out the possibility of using growth rate to separate fungi closely related in form and demonstrated the production of two kinds of secondary spores in culture by *F. fraxineus;* Mounce (1929) made extensive observations on the cultural characteristics of *Fomes pinicola* using malt agar as a standard medium. The most complete study of cultural characteristics was made by Long and Harsch (1918) who tested a number of wood destroying fungi including ten species of *Fomes*. They were particularly interested in the development of sporophores in culture. They originated a number of descriptive terms which have been used in the

[1] Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Botany, The Pennsylvania State College, State College, Pa., August, 1935.

[2] The writer is particularly indebted to Dr. L. O. Overholts, of the Department of Botany, The Pennsylvania State College, for his suggestion of the problem and for his encouragement and aid during the course of the experimentation and during the preparation of this article.

present study. Fritz (1923) demonstrated beyond a doubt that individual species of wood-destroyers produced very characteristic mats in culture and that these characteristic mats were constant for a given species. Snell, Hutchinson and Newton (1928) demonstrated that closely related forms could be separated by growth rate at constant temperatures. Sluzalis (1933) worked out the cultural characteristics of a number of fungi, paying particular attention to the formation of secondary spores. Within the last few years Cartwright (1929, 1934), Verral (1934) and Baxter (1932) have made more or less extensive studies of wood-destroyers, including many species of *Fomes*, in culture, demonstrating the practicability of making identifications of sporophores and rots on the basis of cultural characteristics.

MATERIALS AND METHODS

The purpose of this study has been to work out a suitable system for the identification of *Fomes* species in culture, based upon as simple a procedure as possible. With this in mind, the fungi to be identified were grown on a single kind of agar, for a limited period, in Petri dishes at room temperature. Identification was then based upon macroscopic and microscopic characters. To this basic procedure has been added (1) growth at constant temperatures and (2) reactions obtained with tannic and gallic acid media. At all times, however, emphasis has been placed upon the development of a simple, practical technique which may serve as the basis for future studies on the cultural characteristics of other fungi.

Media. Since personal observations and the experience of others indicate that nothing of great importance is to be gained by growing a fungus on a variety of agars, malt agar, which has been recognized for some time as the standard medium for culturing wood-destroying fungi, was chosen as the medium to be used in this study. Other agars, when used, were merely for the sake of providing supplementary data. The malt agar was prepared by dissolving 20 grams of agar in 1 liter of distilled water either in the autoclave or over an open flame. When the agar was dissolved, 15 grams of Difco malt extract was added and the resulting solution poured into 500 cc. flasks and sterilized for 20 minutes at 15 pounds pressure. The weight of materials and time of sterilization were carefully checked in order to make each batch comparable.

No attempts were made to adjust the pH of the malt agar. Test samples taken at various times indicated a range of from 5.2 to 5.8. Since wood-destroying fungi prefer a slightly acid medium and since the pH may vary within rather wide limits for these fungi, the control of pH within these narrow limits was not considered important.

Tannic and gallic acid media used to differentiate between brown-rot fungi and white-rot fungi were prepared according to Bavendamm's (1928) suggestion, in total concentrations of 0.5 per cent. Since it is impossible to sterilize malt agar containing tannic and gallic acid, the malt agar was prepared as given above using instead of 1000 cc. of water, 800 cc. The proper amounts of the tannic or gallic acids were dissolved in 200 cc. water and sterilized. While still hot the two were mixed together and poured into Petri dishes. When only a few plates of tannic acid medium or gallic acid medium were needed, the malt agar was poured into the dishes and a small amount of the acid dropped in with a scalpel. This short method gave results comparable to more accurate methods since the concentrations of the tannic and gallic acids may vary considerably without affecting results.

No attempt was made to accurately measure the amount of agar used in Petri dish cultures. A thick layer, however, was poured into each dish, considerably in excess of the amount actually required by the fungus, in order to make sure that moisture content would not be reduced appreciably in the 14 days that the dishes were in use.

For stock cultures, 18 mm. test tubes were used. These tubes were filled with 15 cc. of agar and given a comparatively short slant in order to reduce the surface exposed to air. For subcultures a longer slant was used, as here the object was to secure a surface from which inoculating material might be taken rather than to prevent drying of the agar.

Treatment of cultures. The stock cultures were kept in racks in a cupboard where they received but weak diffused light. These cultures were renewed every four months.

Whenever inoculations of Petri dishes were to be made, subcultures were started from 2 to 3 weeks in advance. These subcultures were usually made in Petri dishes although long-slanted test tubes were also used.

In order to secure the most constant results, the following procedure was adopted. Material for the inoculum was taken from near the outer margin of the mats, care being taken to include some of the superficial hyphae as well as the submerged hyphae, the size of the piece being maintained as constant as possible, about 3 mm. square. This was placed with the mycelium side down upon the agar and gently patted in order to bring it into close contact. All transfers were made in a small room with doors and windows closed. No precautions were observed other than mopping up thoroughly with mercuric chloride just prior to transferring, and confining transfer work to the early hours before any sweeping or general moving about of people had stirred up the dust. Very few contaminations resulted and these were never serious enough to interfere with the work.

Cultures to be grown at room temperature were left on the desk in the same room used for transfer work. This room was on the southwest side of the building and had one window. The distance from the window varied from 10 to 12 feet. The shades were adjusted to provide diffused light at all times, although no attempt was made to keep the amount of light constant. Cultures grown at constant temperatures were kept in complete darkness.

In order to eliminate variable results at higher temperatures when working with constant temperature ovens, cultures to be incubated at 36° C were kept at room temperature for 48 hours prior to being placed in the oven, those at 31°, 24 hours, those at 21° and 26° were placed there directly after inoculating. At the higher temperatures it was felt advisable to allow the mycelium to make some growth into the agar before being placed under, in many cases, adverse temperatures for growth and development.

Recording data. Data as to rate of growth, appearance of mat, color, and microscopic features were taken from Petri dish cultures at intervals of seven and fourteen days. Records for test tube cultures were taken at one, two, four and eight weeks. Observations as to macroscopic and microscopic features were taken at least three different times, corresponding to different seasons of the year for each species, and the final description is a composite of the separate descriptions. Colors are described in terms of Ridgway's Color Manual.

Photographs of Petri dish cultures were made at intervals of seven days for fast growing species and at fourteen days for slower growing species. These photographs are designed to show the more usual characteristics of the cultures rather than exceptions.

In all cases emphasis is laid upon the fact that the final descriptions are the result of numerous observations made over a period of 18 months. In this way it has been possible to observe variations of individual cultures under conditions that are bound to vary from season to season.

Source of cultures. Most of the cultures used were isolates obtained from sporophore tissue. Some isolates from the rotted wood were also used. Most of these cultures were available from Dr. L. O. Overholts' collection at The Pennsylvania State College; additional ones were secured from Mr. R. W. Davidson, Office of Forest Pathology, Washington, D. C.; Dr. Irene Mounce, Central Experimental Farm, Ottawa, Canada; Dr. J. S. Boyce, Osborn Botanical Laboratory, New Haven, Conn.; Dr. W. H. Snell, Brown University, Providence, R. I. Other collectors have supplied smaller numbers of cultures and additional ones were obtained from time to time by trips into the field. In most cases identification of the sporo-

phores from which cultures were made has been checked by Dr. Overholts.

In all, 31 species of *Fomes* are reported on in this investigation. This includes the several varieties of *F. igniarius* and the one variety of *F. robustus*, as well as the sterile fungus tentatively designated as the *Sterile Fomes*. One hundred and seventy-nine separate isolations of the *Fomes* species have been examined, distributed as follows among the different species: *F. annosus*, 4; *F. applanatus*, 12; *F. Calkinsii*, 4; *F. conchatus*, 5; *F. densus*, 4; *F. Everhartii*, 11; *F. fomentarius*, 12; *F. fraxineus*, 2; *F. fraxinophilus*, 12; *F. fulvus*, 2; *F. geotropus*, 2; *F. igniarius*, 12; *F. igniarius var. laevigatus*, 8; *F. igniarius var. populinus*, 4; *F. juniperinus*, 1; *F. lobatus*, 3; *F. marmoratus*, 3; *F. meliae*, 2; *F. officinalis*, 4; *F. ohiensis*, 1; *F. Pini*, 7; *F. pinicola*, 18; *F. rimosus*, 11; *F. robustus*, 8; *F. robustus var. tsugina*, 4; *F. roseus*, 5; *F. scutellatus*, 5; *F. subroseus*, 9; *F. tenuis*, 2; *F. torulosus*, 2; *Sterile Fomes*, 1. Of the species listed above, to my knowledge, the following have not been cultured previously or at least accounts of their cultural characteristics have not appeared in the literature: *F. Calkinsii; F. conchatus; F. densus; F. geotropus; F. juniperinus; F. lobatus; F. marmoratus; F. meliae; F. ohiensis; F. robustus; F. scutellatus; F. tenuis; F. torulosus* and the *Sterile Fomes*.

DESCRIPTION OF CULTURES

In the descriptions of cultures an attempt has been made to include all variations that may be expected within a given species when grown on malt agar, at room temperature, in diffused light. Where a number of isolations were available for any species, this desideratum is at least approximated. Where only one or two isolations were available, the description can apply only to these isolations and may or may not be characteristic of the species as a whole. The phrase, *Type Culture*, is used to indicate an isolate which exhibits all the diagnostic characteristics of the species in question, and is a valid culture to be used for purposes of comparison. In case other investigators are interested in these *Type Cultures*, transfers of the same will be maintained at The Pennsylvania State College and have been sent to the Division of Forest Pathology, Washington, D. C.

Growth at seven days and fourteen days is expressed as the diameter of the colony in centimeters. The figures given indicate the range which may be expected from cultures grown in diffused light at room temperature. If a culture grew 6 to 7 cm. in seven days its growth has been described as *rapid;* if from 5 to 6 cm., *moderately rapid;* from 3 to 4 cm., *slow;* and from 1 to 3 cm., *very slow*.

Records of growth at constant temperatures are expressed as average colony diameters for cultures kept in total darkness. It is not feasible to

compare growth rates for cultures kept in dark with those maintained in light at the same temperature as light may considerably affect growth rate.

Texture of mat is described by the following terms taken for the most part from Long and Harsch's list:

Appressed: Mycelium which is prostrate on surface of agar.
Raised: Forming a mound on agar as opposed to appressed.
Cottony: Erect, rather long mycelium spreading in all directions.
Downy: Short, fine hairs loosely scattered over surface of mat giving it a downy appearance.
Felty: Matted with intertwined hairs resembling felt.
Woolly: A dense mass of mycelium, consisting of long, tortuous, matted hairs.
Plumose: Tufts of mycelium with a central axis from which short hyphae radiate.
Silky: Long parallel threads of mycelium, more or less prostrate like combed silk
Nodulose: Forming definite nodes or bunches.
Tufted: Forming tufts.
Floccose: Thin, cottony pubescence, collected in very minute tufts.
Pulverulent: Having a powdery or dusty appearance.
Poroid: Covered with pores.
Homogeneous; Having same appearance throughout.
Mat proper: Portion of mat in direct contact with agar.
Central zone: In case mat is divided into definite areas, the portion about center.
Marginal zone: Part of mat surrounding central zone.
Margin proper: Region of advancing growth.
Fimbriate: Possessing a fringed edge.
Submerged hyphae: Hyphae growing in contact with or through agar.
Superficial hyphae: Aerial hyphae usually forming surface of mat.

In case it has been necessary to make intermediate distinctions between two of the above terms, for example between floccose and cottony, then the compound word, *floccose-cottony*, was used.

In case of reaction with tannic acid, a *strong* reaction indicates that the diffusion zone formed is dark brown and extends considerably beyond the margin of the colony; *moderately strong*, means that the zone is lighter brown and diffuses to a lesser extent; *medium*, means that the zone is light brown and ordinarily not extending beyond the margins of the mat; *faint*, indicates that the reaction is light brown, sometimes difficult to distinguish from the color of the mat proper, and found only under the older portions of the mat. Usually strong reactions will appear in at least 48 hours, while medium and faint reactions will require from four to fourteen days to manifest themselves. In case the reaction is *negative* no discoloration of the agar occurs.

If no secondary spores occur, no mention of spores will be found in the description. Structures not mentioned in the descriptions should be considered as lacking.

1. FOMES ANNOSUS (Fries) Cooke. Type Culture: FP-53378-S. Figures 1–3, 99.

Growth rapid, mat 6–8 cm. in 7 days; typical culture white, loose, floccose-cottony to appressed-cottony, homogeneous, azonate, at times much appressed and compacted about center, decidedly pulverulent; more cottony forms vegetative with few conidia, while appressed forms produce conidia in abundance; margin colorless or faintly white, coarsely fimbriate; condiophores very numerous to rare, borne on aerial hyphae with inflated, subglobose tips, 9–15μ diameter, covered with numerous sterigmata; conidia obovoid, 5–8$\times$3.5–6μ; submerged hyphae 3–4 (−8)μ, with cross-walls but no clamps; aerial hyphae 6–8μ, granular with deeply staining contents; growth at constant temperatures, 5.5 cm., 21°; 8.0 cm., 26°; 4.3 cm., 31°; 0 cm., 36°; strong reaction with tannic acid media.

In 14 days mat appressed-floccose-cottony to floccose-cottony, usually azonate, pulverulent about inoculum or over entire surface, white, sometimes slightly cream colored; odor faint to prominent, musty.

Test tube cultures. White to cream colored up to 4 weeks, becoming cinnamon drab to drab in 8 weeks, mat fragile, pulverulent, at times forming poroid areas made up of closely interwoven hyaline hyphae, thick-walled, uniformly 2μ; no basidia; globose, ellipsoid to short cylindric or irregular-shaped, terminal chalmydospores with definite hyaline wall, 9–15$\times$6–12μ, fairly abundant, intermingled with hyaline hyphae.

Distinguishing characters. The production of characteristic conidiophores and conidia separates *Fomes annosus* from all other *Fomes* species reported here.

2. FOMES APPLANATUS (Pers.) Gill. Type Cultures: 17182-S, 17184-S. Figures 4–8, 100.

Growth slow, mat 2.5–4 cm. in 7 days, typically appressed, thin, colorless to faintly white or chalky white, downy or pruinose, fragile, usually slightly zonate, central portion often much compacted, pruinose-pulverulent, white, forming a tough crust on agar in contrast to fragile, thin, marginal portion; margin proper appressed, colorless, even; submerged hyphae 2–4(–5)μ, much branched, with abundant clamps; superficial hyphae 2–3(–4)μ, either resembling submerged hyphae and bearing numerous irregularly divided, staghorn branches, or thick-walled, hyaline; cuticular bodies abundant to rare in compacted central zone, globose, up to 20μ; growth at constant temperatures 4.1 cm., 21°; 5.0 cm., 26°; 5.5 cm., 31°; 1.0 cm., 36°; moderately strong reaction with tannic acid media.

In 14 days mat 5–8 cm. in diameter, appressed, thin, fragile, colorless

to white, usually slightly zonate, downy to pulverulent or pruinose, with compacted white central zone. Unusual forms at times more compacted and much slower growing, or cottony and growing at a faster rate. Cuticular bodies more abundant; odorless.

Test tube cultures. Mat white up to four weeks with numerous yellowish compacted masses of crystalline material common along edge of mat on agar cylinder, tough, at times considerably wrinkled. Deepest colors developed in eight weeks mouse gray and Quaker drab, fruiting body produced in only one instance culture F-496, with few mature basidiospores, truncate, light brown 6–8×4–5μ. On potato-dextrose producing a much wrinkled and contorted mat.

Distinguishing characters. F. applanatus is readily recognized by the presence of staghorn branches on superficial mycelium and by the absence of chlamydospores. *F. fraxineus* which produces staghorn branches on

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Explanation of figures 1–53

Figs. 1–3. *Fomes annosus.* 1, Conidiophore. 2. Chlamydospores from poroid areas. 3. Hyaline hyphae from poroid areas.

Figs. 4–8. *F. applanatus.* 4. Submerged hypha. 5, Superficial hypha. 6. Staghorn branches. 7, Cuticular cells. 8, Basidiospores.

Figs. 9, 10. *F. Calkinsii.* 9, Submerged hypha. 10, Superficial hypha.

Figs. 11, 12. *F. conchatus.* 11, Submerged hypha. 12, Superficial hypha.

Figs. 13, 14. *F. densus.* 13, Submerged hypha. 14, Superficial hypha.

Figs. 15, 16. *F. Everhartii.* 15, Submerged hypha. 16. Superficial hypha.

Figs. 17–19. *F. fomentarius.* 17, Submerged hyphae. 18, Superficial hyphae. 19, Chlamydospore.

Figs. 20–26. *F. fraxineus.* 20, Submerged hypha. 21, Superficial hypha. 22, Staghorn branches. 23, Large chlamydospores. 24, Small chlamydospores. 25, Basidia. 26, Basidiospores.

Figs. 27–30. *F. fraxinophilus.* 27, Submerged hypha. 28, Superficial hypha. 29, Large chlamydospores. 30, Small chlamydospores.

Figs. 31–35. *F. fulvus.* 31, Submerged hypha, 32, Superficial hypha. 33, Incrusted hypha. 34, Basidia and paraphyses. 35, Basidiospores.

Figs. 36–38. *F. geotropus.* 36, Submerged hyphae. 37, Incrusted hypha, 38, Basidia and basidiospores.

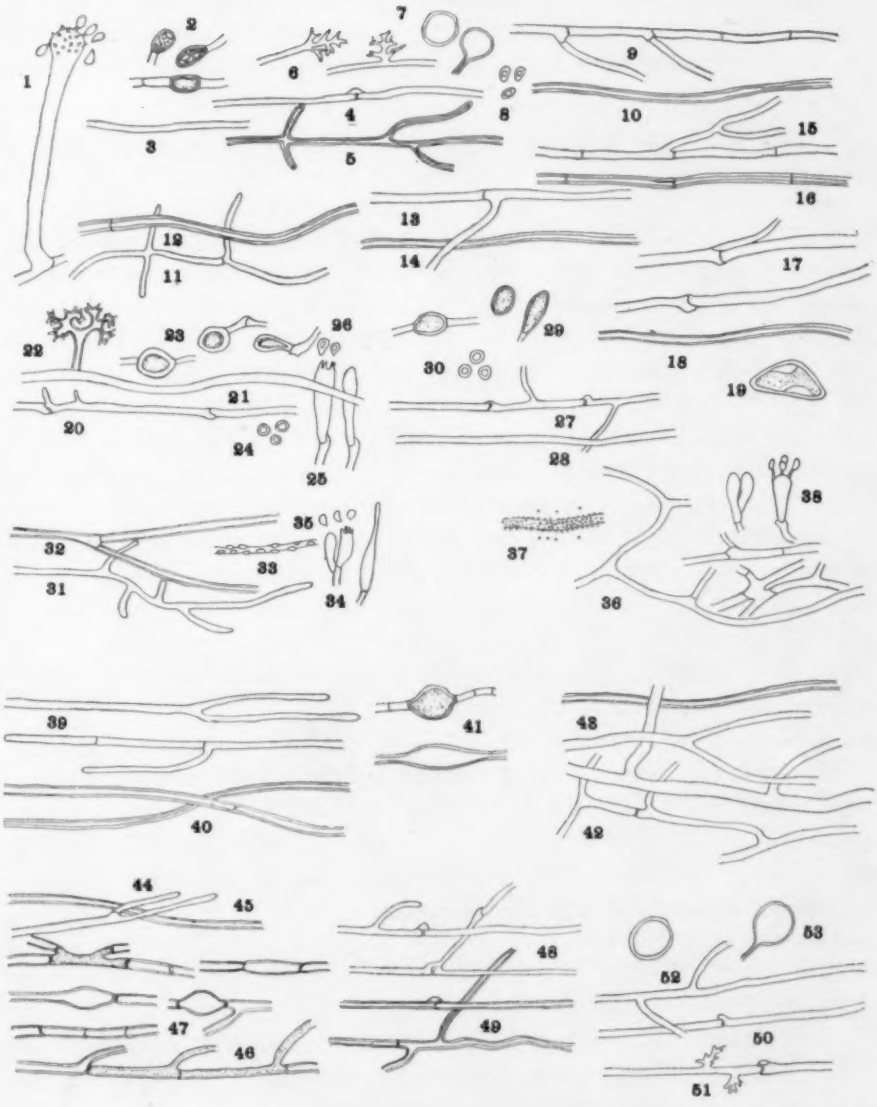
Figs. 39–41. *F. igniarius.* 39, Submerged hyphae. 40, Superficial hyphae. 41, Chlamydospore-like bodies and hyphal swellings.

Figs. 42, 43. *F. igniarius* var. *laevigatus.* 42, Submerged hyphae. 43, Superficial hypha.

Figs. 44–47. *Fomes igniarius* var. *populinus.* 44, Submerged hypha. 45, Superficial hypha. 46, Submerged hyphae broken up into short cells. 47, Hyphal swellings.

Figs. 48, 49. *F. juniperinus.* 48, Submerged hyphae. 49, Superficial hyphae.

Figs. 50–53. *F. lobatus.* 50, Submerged hyphae. 51, Staghorn branches. 52, Superficial hypha. 53, Cuticular cells.



Figs. 1–53. (*See opposite page for legend*)

superficial mycelium produces chlamydospores in abundance. *F. applanatus* cannot be separated with certainty in every instance from *F. lobatus*. However, the latter species usually has a pronounced compacted yellowish to drab central zone and a loose-cottony or only slightly compacted marginal zone.

3. FOMES CALKINSII (Murrill) Sacc. & D. Sacc. Type Culture: 14667-S. Figures 9, 10, 101.

Growth slow, mat 2–3 cm. in 7 days, white at margin, pale orange yellow or occasionally deepening to antimony yellow over central portion, raised, compacted, woolly to felty, often with faint narrow zones; margin white, short cottony, even; submerged hyphae 2–5(–6)μ. with cross-walls but no clamps, hyphae under colored portion of mat thin-walled, brownish with abundant cross-walls spaced at short intervals; superficial hyphae 2–3(–4)μ, thick walled, brown, with few cross walls, little branched; growth at constant temperatures 2.0 cm., 21°; 2.5 cm., 26°; 2.5 cm., 31°; trace, 36°; strong reaction with tannic acid medium.

In fourteen days mat 4–5 cm., white at margin to pale orange yellow and antimony yellow over central portion, raised, compacted, woolly to felty, spongy; margin white short cottony, even; odorless.

Test tube cultures. Mat in four weeks forming definite fruiting pad on slant, tough, heavy, from mustard yellow, through antimony yellow to yellow ochre and buckthorn brown.

Distinguishing characters. F. Calkinsii is difficult to separate from *F. robustus* but the former is more delicate, lighter colored and grows somewhat slower, and becomes more pallid in the dark. *F. Calkinsii* is inhibited entirely at 36° C while *F. densus* is able to make good growth. Similarly *F. Calkinsii* may be separated from *F. conchatus*, *F. Everhartii*, *F. rimosus* and *F. torulosus* on temperature relations.

4. FOMES CONCHATUS (Pers.) Gill. Type culture: 524 Mad. Figures 11, 12, 102.

Growth very slow, mat 1–2 cm. in 7 days, white with at times a yellowish tinge about inoculum; thin, cottony to thick, felty-cottony, too small to possess many distinctive features and consisting of a white cottony, relatively undifferentiated mass of hyphae; margin white, cottony, fimbriate; submerged and superficial hyphae not distinct, 2–5μ, with cross-walls but no clamps; growth at constant temperatures, 1.7 cm., 21°; 2.3 cm., 26°; trace, 31°; 0 cm., 36°; strong reaction with tannic acid medium.

In fourteen days mat 2.5–4 cm., somewhat variable with few dis-

tinctive features, usually raised, compacted felty-cottony about center to more loosely-cottony at margin, compacted central zone varying in color from fawn to buckthorn brown, often partially obscured by white to yellowish superficial covering; marginal zone chalky white; margin proper white, cottony, fimbriate; colored superficial hyphae uniformly 2–5μ, thick-walled, non-staining, branched, with abundant cross-walls, yellow color of superficial hyphae diffusing strongly in KOH solution; globose vesicular bodies common in some cultures, rare in others, mostly terminal, up to 20μ in diameter; odorless.

Test tube cultures. In 4 weeks mat forming pad on slant, usually uneven on agar cylinder, varying considerably in color from warm buff to yellow ochre and buckthorn brown.

Distinguishing characters. Fomes conchatus is one of the slower growing colored species of *Fomes* with a restricted temperature range being almost completely inhibited at 31° C. It can be distinguished from *F. Pini* by color and temperature relations and from *F. igniarius var. populinus* by more rapid growth and by its chalky white mat in early stages and absence of odor.

5. FOMES DENSUS Lloyd. Type culture: FP-52033-S. Figures 13, 14, 103.

Growth slow, mat in 7 days 2–3 cm., straw yellow at margin, gradually deepening to mustard yellow and primuline yellow over inoculum, raised, loosely felty-cottony to woolly, at times compacted, homogeneous, azonate; margin proper even, colorless; submerged hyphae 3–5(–7)μ, with few cross-walls and no clamps; superficial hyphae moderately thick-walled, yellowish 2–4μ; growth at constant temperatures 2.0 cm., 21°; 2.6 cm., 26°; 4.7 cm., 31°; 2.0 cm., 36°; strong reaction with tannic acid medium.

In 14 days mat 4.5–6 cm., dense, closely compacted, woolly to felty-cottony, nodulose, raised considerably above agar, homogeneous, straw yellow at margin to mustard yellow and primuline yellow at center, superficial raised portion tough, spongy, separating freely from agar, turning ochraceous buff when touched with KOH, later light brown; odorless.

Test tube cultures. In 4 weeks mat raised, compacted, with definite fruiting pad on slant, homogeneous in color and texture, tough antimony yellow and yellow ochre.

Distinguishing characters. F. densus does not separate readily from *F. robustus* and *F. Calkinsii.* However, it does show a considerably faster growth rate at 31° than either of these and the yellow color which rarely deepens to antimony yellow is quite distinctive, since both *F. Calkinsii* and *F. robustus* become yellow ochre or darker in Petri dish cultures in 14

days. Both *F. Calkinsii* and *F. robustus* turn brown when touched with KOH without going through an intermediate ochraceous buff stage.

6. FOMES EVERHARTII (Ell. & Gall.) von Schrenk Type culture: 17412-S. Figures 15, 16, 104.

Growth slow, mat 2–3 cm. in 7 days, dark, approximately Sudan brown, fragile, not separating from agar, superficial covering thin to dense, ochraceous buff and antimony yellow at center to pale yellow or white at margin, often with a greenish yellow sheen, silky to silky-cottony, partially to completely obscuring color of mat proper; margin colorless to white, appressed to cottony, fimbriate; submerged hyphae from growing tip 2–4(–6)μ, thin-walled, staining readily with eosin, few cross-walls and no clamps, from mat proper 2–4(–6)μ, thick-walled with brownish content and with abundant cross-walls; superficial hyphae, 2–3(–4)μ, thin to moderately thick-walled, darkening in KOH; growth at constant temperatures 2.0 cm., 21°; 3.2 cm., 26°; 3.6 cm., 31°; 1.5 cm., 36°; strong reaction with tannic acid medium.

In 14 days mat 4–6 cm., mat proper Sudan brown, closely compacted to agar, rather thick; superficial covering antimony yellow or pale yellow at center to white at margin, silky to silky-cottony, partially to completely obscuring color of mat proper; odorless.

Test tube cultures. Mat in 4 weeks Dresden brown to mummy brown, thick, clinging to agar, superficial covering silky-cottony, antimony yellow to yellow ochre, becoming faded with age, finally warm buff through cinnamon buff to honey yellow.

Distinguishing characters. In general *F. Everhartii* if grown in light can readily be distinguished from *F. igniarius*, *F. rimosus* and *F. densus* by dark mat proper and by silky superficial covering, often with a greenish tint, and usually turning vinaceous when rubbed with KOH.

7. FOMES FOMENTARIUS (L.) Gill. Type culture: 17041-S. Figures 17–19, 105.

Growth rapid, mat in 7 days 6–7 cm., white to cream colored or light ochraceous buff and light ochraceous salmon, ochraceous color localized about inoculum or covering entire surface, azonate, homogeneous, closely appressed-cottony to appressed-velvety, forming a distinct, somewhat tough film on agar; margin white or colorless, fimbriate; chlamydospores rare or absent, ellipsoid, ovoid, or irregular, 20–28$\times$12–18μ, with thick hyaline wall; submerged hyphae, 3–5μ, with abundant clamps; superficial hyphae 2–4μ, hyaline or colored, thick-walled, uniform, without clamps; growth at constant temperatures 5.0 cm., 21°; 7.0 cm., 26°; 6.4 cm., 31°; trace 36°; strong reaction with tannic acid medium.

In 14 days mat solidly light ochraceous buff to buckthorn brown or with irregular white or cream colored areas, rarely entire mat white or cream colored, forming tough, thin film on agar, surface appressed-velvety; margin against glass often forming distinct, yellowish papillae which contain crystalline material; odor indistinct, sweetish.

Test tube cultures. In 4 weeks mat appressed-velvety, cream colored to buckthorn brown, with numerous yellowish papillae against glass later darkening to cinnamon brown and Dresden brown.

Distinguishing characters. F. fomentarius can readily be distinguished in culture by the thin, velvety, appressed mat and by lack of pore surfaces and vesicular bodies. The only species that it superficially resembles, *F. marmoratus*, produces conspicuous cuticular or vesicular bodies. The yellowish papillae against the test tube glass is characteristic of *F. fomentarius*.

8. FOMES FRAXINEUS (Bull. ex Fries) Cooke-Type culture: FP-57061-S. Figures 20–26, 106.

Growth moderately rapid, mat 5–6 cm. in 7 days, white, homogeneous, appressed, pulverulent to granular about inoculum to appressed coarse cottony near margin; margin proper faintly white, very coarsely fimbriate; chlamydospores very numerous, mostly globose to lemon-shaped, 10–15 μ in diameter, when mature with heavy hyaline wall up to 4μ thick; occasional cultures exhibit few to many clavate, immature basidia which resemble irregular-shaped chlamydospores; submerged hyphae up to 4μ, with abundant clamps; superficial hyphae 2–4μ, hyaline, thick-walled, often bearing numerous dichotomously divided staghorn branches from tips and sides; growth at constant temperatures 4.0 cm., 21°; 6.5 cm., 26°; 8.5 cm., 31°; 2.5 cm., 36°; strong reaction with tannic acid medium.

In 14 days mat white, appressed, pulverulent to granular or appressed, coarse cottony, with raised velvety patches and at times with faint to prominent poroid areas, white to faintly vinaceous; chlamydospores of type described at 7 days very numerous, in addition poroid areas contain small, subglobose chlamydospores 6–8$\times$5–6μ; immature basidia common in poroid areas, mature basidia rare, clavate 8–10μ diameter; basidiospores ellipsoid 5–7$\times$3–4μ; odorless.

Test tube cultures. Mat white with sporophore developing on lower half of slant in 4 weeks, pores deep purplish vinaceous, producing subglobose chlamydospores in great abundance.

Distinguishing characters. F. fraxineus may be distinguished from *F. fraxinophilus*, which it resembles very closely in appearance, by heavy walled chlamydospores, by the staghorn branches on superficial mycelium,

and by the vinaceous color of the pore surface as produced in tube cultures. It may be distinguished from *F. applanatus* and *F. lobatus* by production of chlamydospores and by the formation of pores in tube cultures.

9. FOMES FRAXINOPHILUS (Peck) Sacc. Type culture: 15159-R. Figures 27–30, 107.

Growth moderately rapid, mat 4–5 cm. in 7 days, white, appressed nodulose-cottony to appressed velvety-cottony, often forming poroid areas, tough, peeling readily from agar; marginal zone broad, fragile, colorless, appressed-cottony; margin proper colorless, fimbriate; chlamydospores abundant, globose to clavate or irregular, 8–14×5–8μ, rather thin-walled; submerged hyphae 1–4μ, clamps abundant; superficial hyphae 1–3μ, thick-walled, smooth, little branched; growth at constant temperatures 3.5 cm., 21°; 6.0 cm., 26°; 7.0 cm., 31°; 5.0 cm., 36°; moderately strong reaction with tannic acid medium.

In 14 days mat white, finely nodulose-cottony to velvety-cottony, often with well developed poroid areas over central zone, azonate, tough, peeling readily from agar; poroid areas often producing abundant globose to subglobose, secondary spores, 6–8×5–6μ, thick-walled, hyaline, produced on short branches of superficial hyphae; immature basidia also produced in poroid areas, no mature basidiospores noted; odor faint, mushroom-like.

Test tube cultures. In 4 weeks producing wood colored pores mostly on upper part of slant, rest of mat white, pores containing numerous small secondary spores.

Distinguishing characters. F. fraxinophilus may be distinguished from *F. fraxineus* by lack of staghorn branches on superficial mycelium and by wood colored pores. *F. fraxinophilus* may be distinguished from all other *Fomes* having a white mat by the production of pores in tube cultures and by the small, subglobose secondary spores.

10. FOMES FULVUS Scop. ex. Gill. Type culture: 16870-S. Figures 31–35, 108.

Growth slow to moderately rapid, mat 3.5–5 cm. in 7 days, thin, appressed long-cottony, strongly to faintly zoned, with superficial mycelium orientated toward center, surface about inoculum sometimes covered with scattered patches of compacted, raised, hardened mycelium, which develop into poroid areas, but in other isolates no evidence of poroid areas, in general ochraceous buff to cinnamon buff with compacted, nodulose patches darkening to buckthorn brown; marginal zone greyish white

coarse-cottony; margin proper colorless, even; no secondary spores; immature basidia abundant in poroid areas; submerged hyphae 2–6μ, without clamps but with cross-walls, gradually grading into white superficial hyphae with definite hyaline wall, often incrusted with large smooth crystals; brown superficial hyphae 3–4μ, heavy walled, darkening in KOH; growth at constant temperatures 4.5 cm., 21°; 5.0 cm., 26°; 4.5 cm., 31°; 2.5 cm., 36°; moderately strong reaction with tannic acid medium.

In 14 days mat 8–9 cm., appearance same as at 7 days, poroid areas more numerous giving nodulose to pulverulent appearance to mat, basic portion ochraceous buff to cinnamon buff, poroid places buckthorn brown or darker; secondary spores none; mature basidia clavate to short cylindric, 10–15$\times$3.5μ, usually forming definite hymenial layer containing narrow paraphyses extending up to 20μ and occasionally brown setae; basidiospores ellipsoid to allantoid, slightly flattened on one side, 3–4$\times$2–3μ; odorless.

Test tube cultures. Mat thin, appressed, with hard appearing surface or more cottony, ochraceous buff to antimony yellow and yellow buff with poroid areas or remaining undifferentiated.

Distinguishing characters. F. fulvus may be readily identified by production of fruiting structures in culture; when these are lacking, however, growth rate and temperature relations must be relied upon to separate it from other rapid growing *Fomes* species.

11. FOMES GEOTROPUS Cooke. Type cultures: FP-55521-S, FP-55558-S Figures 36–38, 109.

Growth very rapid, mat 7 cm. in 4 days, white, closely appressed cottony to loosely cottony, homogeneous, very fragile, azonate, closely adhering to agar; secondary spores none; immature basidia at times numerous, often lacking; submerged hyphae small, 2–3(–4)μ, without clamps; superficial hyphae up to 7μ, without clamps, staining deeply with eosin, often incrusted; growth at constant temperatures in 7 days, 9.0 cm., 21°; 15 cm., 26°; 15 cm., 31°; 9 cm., 36°; faint reaction with tannic acid medium.

In 14 days mat white, either closely appressed, pulverulent, homogeneous, with tendency to form sporophores, or loosely cottony, tufted, without sporophores; mature basidia abundant; basidiospores ellipsoid, 4–5$\times$3–4μ; odor faint, mushroom-like.

Test tube cultures. In 4 weeks, white, appressed or loosely cottony usually portion on agar producing yellowish areas containing crystalline material.

Distinguishing characters. F. geotropus may be distinguished from all

other *Fomes* species that produce a white mat in culture on growth rate alone, forming a mat 10–12 cm. in diameter in 7 days at room temperature.

12. FOMES IGNIARIUS (L. ex Fries) Gill. Type cultures: 17171-S, 17355-S. Figures 39–41, 110.

Growth slow, mat 2–3 cm. in 7 days, raised, compacted woolly to felty or loosely felty-cottony, at times nodulose or ridged, definitely two-zoned, central zone ochraceous buff, through clay color to buckthorn and Dresden brown, marginal zone narrow to broad, chalky white; margin proper colorless to white, somewhat fimbriate; submerged hyphae 2–5μ, without clamps; superficial hyphae of two kinds, either colorless up to 7μ, with slightly thickened hyaline wall and deeply staining contents, or colored, with thick hyaline wall, non-staining, 2–4μ, with few cross-walls; growth at constant temperatures 3.5 cm., 21°; 4.2 cm., 26°; 3.1 cm., 31°; 1.5 cm., 36°; strong reaction with tannic acid medium.

In 14 days mat 5–6 cm., raised, loosely felty to compacted fine-woolly, two-zoned nature less pronounced, central portion yellow ochre to buckthorn brown and often as dark as Sudan brown and antique brown; color often toned down by whitish to yellowish superficial covering, tough, peeling readily from agar in thick felted sheets leaving whitish film attached to agar; secondary spores questionable, occasional globose to irregular thick-walled chlamydospore-like bodies produced up to 30μ in diameter; odorless.

Test tube cultures. Fruiting pad formed on agar slant, usually fine woolly, warm buff to ochraceous buff, rest of mat buckthorn brown to Dresden brown, often with alternating light and dark bands on agar cylinder.

Distinguishing characters. It is possible to confuse *F. igniarius* with certain other colored species such as *F. robustus, F. rimosus, F. torulosus and F. densus.* However, *F. igniarius* possesses a more distinctly two-zoned nature and peels more readily from the agar leaving a whitish film attached. It differs from *F. igniarius* var. *laevigatus* by growing at a much slower rate and in possessing no odor.

13. FOMES IGNIARIUS var. LAEVIGATUS Fries. Overh. Type culture: 17359-S. Figures 42, 43, 111.

Growth rapid, mat 4.5–6 cm. in 7 days, usually two-zoned, central zone closely compacted, felty to felty-cottony, moderately thick, homogeneous, peeling evenly and cleanly from underlying agar, ochraceous tawny to buckthorn brown, marginal zone appressed cottony to woolly, changing from white on outer limits to yellowish on inner, adhering to agar, thin; margin proper white, thin, scraggly, coarsely fimbriate; sub-

merged hyphae, 3–7μ, cross-walls common, clamps none or rare; superficial hyphae of two kinds, white, staining in eosin, with moderately thick hyaline wall 3–7μ, or brown, thick-walled, non-staining, 1.5–3(–4)μ, little branched and with few cross-walls; growth at constant temperatures 4.7 cm., 21°; 6.5 cm., 26°; 6.0 cm., 31°; 2.5 cm., 36°; strong reaction with tannic acid medium.

In 14 days mat homogeneous, evenly ochraceous tawny to buckthorn brown, closely compacted, appressed, nodulose, felty to felty-cottony, separating cleanly from underlying agar in thick felted sheets; odor sweetish, resembling wintergreen, most pronounced when cultures are grown in the dark.

Test tube cultures. In 4 weeks, mat felty-cottony, evenly buckthorn brown to Dresden brown, no pad formed.

Distinguishing characters. F. igniarius var. *laevigatus* may be distinguished from *F. igniarius* by its more rapid rate of growth and by its characteristic odor when grown in the dark.

14. FOMES IGNIARIUS var. NIGRICANS Fries.

The various isolates designated *F. igniarius* var. *nigricans* showed no close agreement as to cultural characters. Some were readily recognizable as *F. igniarius* var. *laevigatus* while others differed but slightly from the typical *igniarius*. Until a study can be made of more isolates of the resupinate form of *igniarius* called the *nigricans* variety, it will be impossible to state whether or not a distinct species exists recognizable by cultural characteristics.

15. **Fomes igniarius** var. **populinus** (Neuman) n. comb. Type culture: 16431-S. Figures 44–47, 112.

Growth extremely slow, mat 1–2 cm. in 7 days, variable, compacted, appressed vinaceous brown and haematite red to Brussels brown and raw umber, individual isolates either solidly one color or a combination of several, usually with a fairly distinct, narrow, white to yellowish border; surface of mat covered with a thin to heavy, short-velvety to downy, superficial covering which may be same color as mat proper or somewhat lighter; margin proper narrow, colorless, very closely appressed, even; mat usually producing heavy brown diffusion zone in ordinary malt agar; globose to ellipsoid or irregular-shaped swellings fairly common in some cultures on colored hyphae; hyphae in general show a gradual gradation from submerged thin-walled colorless type to brown superficial type, submerged 3–4(–7)μ, thin-walled, many cross-walls, much branched, often broken up into short lengths by empty cells, older hyphae yellowish in ap-

pearance, non-staining with ellipsoid swellings, empty hyaline hyphae also common, often showing constrictions at cross-walls; on surface of cultures short lengths of dark, thick-walled hyphae common in some isolations rare in others, 3–4μ; growth at constant temperatures 1.5 cm., 21°; 2.0 cm., 26°; 1.0 cm., 31°; trace 36°; strong reaction with tannic acid medium.

In 14 days mat 1.5–4 cm., variable, closely appressed, compacted, raw sepia to Brussels brown with at times a distinct lighter colored marginal zone, at times appearing much as reduced forms of *F. igniarius* with pronounced two-zoned mat; odor very pronounced, characteristic, sweet, agreeable.

Test tube cultures. Poor growth in 4 weeks, mat much appressed, dark, with strong diffusion zone in agar.

Distinguishing characters. On growth rate alone *F. igniarius* var. *populinus* can be confused only with *F. robustus* var. *tsugina* and possibly with *F. conchatus*. Odor is sufficient to distinguish it from the ones mentioned while resemblance to the typical *igniarius* will help to distinguish the individual isolations that grow at a faster rate than the usual *Populus* form.

16. FOMES JUNIPERINUS (Schrenk) Sacc. & Sydow Description based on F-3765. Figures 48, 49.

Growth extremely slow, mat 1 cm. in 7 days, homogeneous, appressed, fine woolly-cottony, olive buff to deep olive buff over inoculum, white at margin, usually producing strong diffusion zone in agar; submerged hyphae and colorless superficial hyphae 2–4μ, clamps abundant; yellow superficial hyphae 2–3μ, non-staining, darkening in KOH, thick-walled and with clamps; constant temperature studies unsatisfactory as fungus makes little measurable growth in 7 days, growth checked above 31°; strong reaction with tannic acid medium.

In 14 days mat 1.5 cm., center olive buff to deep olive buff, marginal portions white or yellowish; odorless.

Test tube cultures. In 4 weeks producing a fine woolly to velvety, olive buff to antimony yellow mat, fragile, homogeneous.

Distinguishing characters. Fomes juniperinus as here described may be separated readily from all other *Fomes* producing clamps by its fine woolly mat, by its olive buff color, and by its slow rate of growth.

17. FOMES LOBATUS (Schw.) Cooke. Type culture: FP-57032-S. Figures 50–53, 113.

Growth slow, mat 2.5–4 cm. in 7 days, definitely two-zoned, central zone appressed, white to yellowish, compacted, often wrinkled, pulveru-

lent to pruinose; marginal zone white, loosely cottony with radiating growth lines in sharp contrast to amorphous central zone; margin proper colorless to faintly white, appressed, fimbriate; submerged hyphae 2–4 (–5)μ, clamps common; superficial hyphae 2–3μ, hyaline, non-staining; cuticular cells common in central zone; staghorn branches on surface hyphae, hyaline, readily broken off; growth at constant temperatures 3.5 cm., 21°; 5.0 cm., 26°; 4.0 cm., 31°; 1.0 cm., 36°; strong reaction with tannic acid medium.

In 14 days mat 7–8 cm.; central zone making up most of mat, appressed, much compacted, often wrinkled and furrowed, pulverulent to pruinose or at times covered with scattered patches of short cottony mycelium, solidly avellaneous to buffy brown or only somewhat yellowish in scattered areas that are usually grouped into one or more concentric rings; marginal zone white, cottony; cuticular cells very abundant in central zone; odor variable, faint to prominent, difficult to describe.

Test tube cultures. In 4 weeks mat changing from white to yellowish or cinnamon drab on slant, roughened, granular, pulverulent, the portion on agar cylinder irregularly nodulose, cottony, often yellowed with granular masses containing crystals. Deepest colors reached in 8 weeks cinnamon drab to buffy brown.

Distinguishing characters. Fomes lobatus may be distinguished from all other *Fomes* species producing staghorn branches on superficial mycelium by the dense compacted yellowish to drab central zone containing cuticular cells and by the cottony radiating, white, marginal zone.

18. FOMES MARMORATUS(Berk. & Curt.) Cooke. Type Culture: FP-57058-S. Figures 54–57, 114.

Growth slow, mat 3–4 cm. in 7 days, appressed, thin, faintly to prominently zonate, somewhat cottony with radiating growth lines, uniformly faintly white to somewhat ochraceous or deepening to cinnamon buff and cinnamon, usually with appressed, wrinkled, compacted area about inoculum, cinnamon buff to sayal brown, either localized or extensive, pulverulent, whitish in spots; oidia rare to fairly abundant, in chains of few to several, thin-walled, 3–5μ in diameter and in lengths up to 35μ; cuticular cells abundant in compacted areas, thin-walled, colored when mature, up to 25μ diameter; submerged hyphae, 2–4(–6)μ, clamps abundant; superficial hyphae uniformly 2–3(–4)μ, thick-walled, colored or hyaline; growth at constant temperatures 3.8 cm., 21°; 5.7 cm., 26°; 6.5 cm., 31°; 4.6 cm., 36°; moderately strong reaction with tannic acid medium.

In 14 days mat 7–8 cm., appressed, thin, zonate, cottony, faintly white through pinkish buff to cinnamon buff and cinnamon, either evenly

colored or deeper colors confined to irregular patches, center wrinkled, appressed pulverulent, cinnamon buff to sayal brown; no distinct odor.

Test tube cultures. In 4 weeks upper part of mat appressed, pulverulent, compacted, Dresden brown to raw umber, separated from white or smoky portion on agar cylinder by a dark, raised line.

Distinguishing characters. F. marmoratus is the only one of the brown colored species of *Fomes* to produce cuticular bodies in culture. Presence of oidia also very characteristic.

19. FOMES MELIAE (Underwood) Murrill. Description based on FP-50236-R. Figures 58–60, 115.

Growth slow, mat 3.5–5 cm. in 7 days, thick, homogeneous, azonate, white, fine velvety-cottony; margin white to colorless, coarsely fimbriate; chlamydospores none or few produced in 7 days, lemon-shaped or ellipsoid 10–15×6–9μ; submerged hyphae 2–4μ, clamps abundant; superficial hyphae, 2–3(–4)μ, hyaline, non-staining; growth at constant temperatures 3.5 cm., 21°; 4.5 cm., 26°; 7.0 cm., 36°; 8.0 cm., 36°; no reaction with tannic acid medium.

Explanation of figures 54–98

Figs. 54–57. *F. marmoratus.* 54, Submerged hyphae. 55, Superficial hypha. 56, Oidia. 57, Cuticular cells.

Figs. 58–60. *F. meliae.* 58, Submerged hyphae. 59, Superficial hypha. 60, Chlamydospores.

Figs. 61–63. *F. officinalis.* 61, Superficial hypha. 62, Submerged hyphae. 63, Chlamydospores.

Fig. 64. *F. ohiensis.* Hyphae.

Figs. 65–67. *F. Pini.* 65, Submerged hypha. 66, Superficial hypha. 67, Hyphal swellings.

Figs. 68–71. *F. pinicola.* 68, Submerged hyphae. 69, Superficial hypha. 70, Chlamydospores. 71, Basidiospores.

Figs. 72, 73. *F. rimosus.* 72, Submerged hyphae. 73, Superficial hypha.

Figs. 74, 75. *F. robustus.* 74, Submerged hyphae. 75, Superficial hypha.

Figs. 76–80. *F. roseus.* 76, Submerged hyphae. 77, Superficial hypha. 78, Chlamydospores. 79, Basidia. 80, Basidiospores.

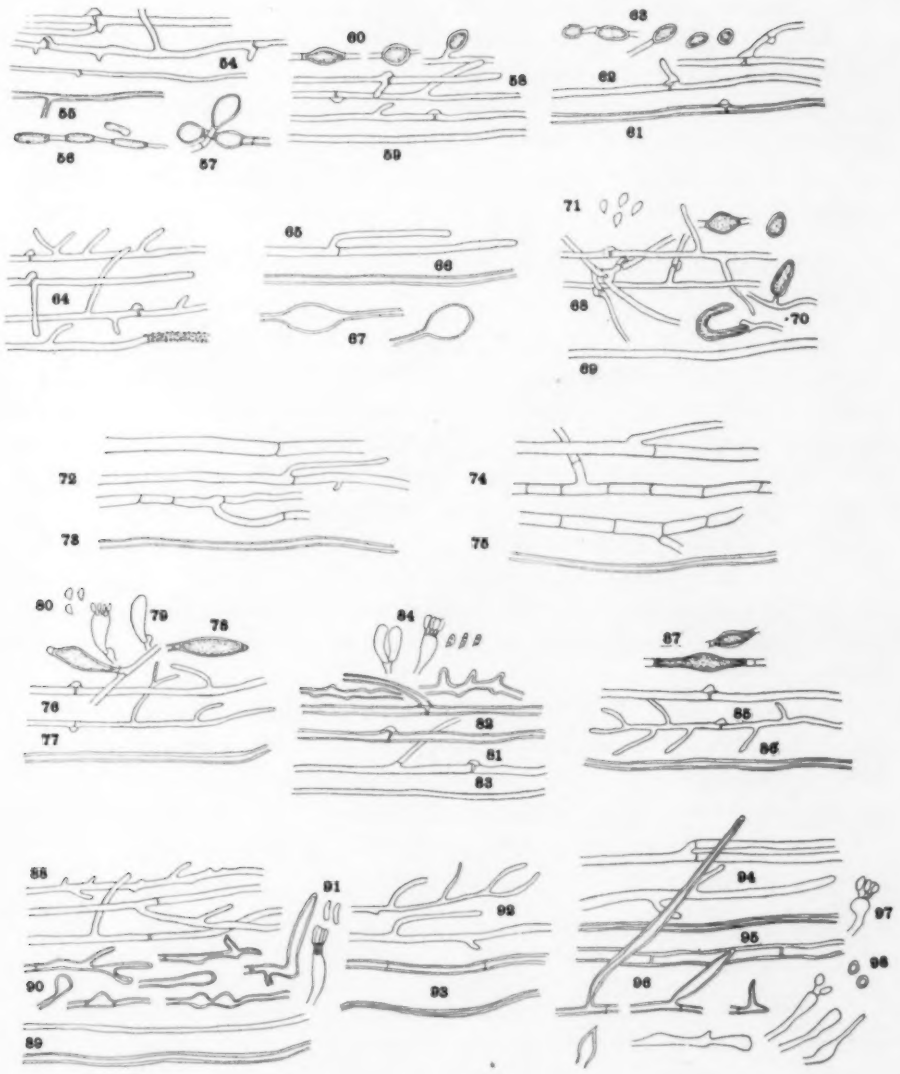
Figs. 81–84. *F. scutellatus.* 81, Submerged hypha. 82, Superficial hyphae. 83, Smaller superficial hypha. 84, Basidia and basidiospores.

Figs. 85–87. *F. subroseus.* 85, Submerged hyphae. 86, Superficial hypha. 87, Chlamydospores.

Figs. 88–91. *F. tenuis.* 88, Submerged hyphae. 89, Superficial hyphae. 90, Irregular hyphal lengths and setae. 91, Basidia and basidiospores.

Figs. 92, 93. *F. torulosus.* 92, Submerged hyphae. 93, Superficial hyphae.

Figs. 94–98. *Sterile Fomes.* 94, Submerged hyphae. 95, Superficial hypha. 96, Setae from surface of mat. 97, Basidia. 98, Basidiospores.



Figs. 54–98. (*See opposite page for legend*)

In 14 days mat 8 cm. or more, white, finely velvety-cottony, homogeneous, azonate, *forming a heavy gelatinous film on agar;* chlamydospores more common; strong mushroom-like odor.

Test tube cultures. In 4 weeks mat white, homogeneous, at times producing fruiting body and mature spores.

Distinguishing characters. F. meliae differs from all other *Fomes* species here reported except *F. geotropus* in that its optimum temperature is around 36°, and by the tough, gelatinous nature of the film formed on the surface of the agar.

20. FOMES OFFICINALIS (Vill. ex Fries) Faull. Type culture: F-1276. Figures 61–63, 116.

Growth very slow, mat 1–2 cm. in 7 days, white, raised woolly to woolly-cottony, homogeneous; azonate, fragile; margin colorless to faintly white, narrow to wide, somewhat appressed fimbriate; chlamydospores numerous, globose to ellipsoid 6–15μ, with irregular shaped ones much larger; submerged hyphae 2–5μ, clamps abundant; superficial hyphae very similar to submerged hyphae but with slightly thicker walls, 2–5μ; growth at constant temperatures 1.0 cm., 21°; 2.2 cm., 26°; 1.5 cm., 31°; 0 cm., 36°; no reaction with tannic acid medium.

In 14 days mat 2–4 cm., white, raised woolly to woolly-cottony, homogeneous, azonate, fragile; chlamydospores more numerous than at 7 days; odorless.

Test tube cultures. In 4 weeks, mat white, nodulose, cottony to floccose on slant, fragile, powdery, rather loosely floccose-cottony on agar cylinder, becoming in time a powdery mass of chlamydospores.

Distinguishing characters. Fomes officinalis may be distinguished from all other species not reacting with tannic acid by the slow rate of growth and the production of chlamydospores.

21. FOMES OHIENSIS (Berk.) Murrill. Description based on FP-52020-S. Figures 64, 117.

Growth very slow, mat 1–2 cm. in 7 days, white or colorless, closely appressed with at times a glazed appearance; margin colorless, even; hyphae essentially of one kind, up to 4μ, with abundant clamps; growth too slow to secure satisfactory data 1–2 cm. in 7 days at 21° and 26°, little or no growth at 31° and 36°; strong reaction with tannic acid medium.

In 14 days mat 2–3 cm., dense, very closely appressed, milky white, sometimes cream colored, finely granular to the eye, central portion at times pulverulent, azonate with at times a marked ring paralleling margin; chlamydospores few or none; not abundant until culture has aged con-

siderably; hyphae about center heavily incrusted; odor faint, mushroom-like.

Test tube cultures. In 4 weeks mat chalky white to cream colored, appressed, pulverulent, compacted, marginal portion on agar cylinder very finely floccose-cottony.

Distinguishing characters. Fomes ohiensis may be distinguished from all other reactors with tannic acid medium by its extremely slow rate of growth and by its white color. *F. tenuis* bears some resemblance but becomes definitely colored in 14 days, while *F. conchatus* does not have clamps as does *F. ohiensis.*

22. FOMES PINI (Thore) Lloyd. Type culture: 17553-S. Figures 65–67, 118.

Growth slow, mat 2–3 cm. in 7 days, cultures divided into two forms, one form producing a loose-cottony, raised mat, yellow to ochraceous buff, the other form producing a thick, felted, appressed mat, ochraceous buff, through antimony yellow to yellow ochre; margin proper even, colorless; submerged hyphae 2.5–4(–5)μ, no clamps; superficial hyphae of two kinds, either white, staining heavily in eosin, with moderately thick hyaline walls, or colored, thick-walled, 2.5–4μ, often with irregular-shaped swellings which resemble chlamydospores, *in KOH the color diffuses readily turning the solution golden-brown;* growth at constant temperatures 1.7 cm., 21°; 2.5 cm., 26°; 2.0 cm., 31°; trace, 36°; moderately strong reaction with tannic acid medium.

In 14 days mat 3–4 cm., two forms persisting, not much change in appearance; increase in number of hyphal swellings; odor faint, musty.

Test tube cultures. In 4 weeks, mat felty-cottony, nodulose, uneven, appressed in spots, cottony pad at times formed on slant, varying from ochraceous buff to yellow ochre and buckthorn brown, pad usually lighter, warm buff to ochraceous buff.

Distinguishing characters. F. Pini may be distinguished from all other colored species of *Fomes* except *F. conchatus* by the golden yellow color imparted by yellow superficial hyphae to KOH; however, variations in appearance of mat and temperature relations are sufficient to separate *F. Pini* from *F. conchatus.*

23. FOMES PINICOLA (Sw.) Cooke. Type cultures: 16433-S, 15348-S. Figures 68–71, 119.

Growth very variable, mat 3.5–6 cm. in 7 days, either thin and loosely floccose-cottony or rather felty, white, homogeneous; margin thin, colorless or faintly white, coarsely fimbriate; chlamydospores few to numerous, globose to ellipsoid or irregular, 7–12×5–9μ; submerged hyphae

2–5μ, clamps abundant; superficial hyphae 2–4μ, thick-walled, hyaline, without clamps; growth at constant temperatures 4.3 cm., 21°; 6.0 cm., 26°; 7.6 cm., 31°; trace to 1.0 cm., 36°; no reaction with tannic acid medium.

In 14 days mat 7 cm. or more, white, appressed-felty or floccose-cottony to loosely floccose-cottony, forming fairly tough film on agar; submerged hyphae with tendency to break up into irregular shapes not definite enough to be classed as oidia; odor distinct, mushroom-like.

Test tube cultures. In 4 weeks, mat white with tendency to verge toward pink or cream color, very fragile, appressed on slant; finely cottony to floccose-cottony on agar cylinder, some isolates forming fruiting structures.

Distinguishing characters. F. pinicola can be confused with several of the non-reactors on tannic acid media, namely *F. meliae*, *F. subroseus* and *F. roseus*. From *F. meliae* it may be separated on temperature basis since *F. pinicola* barely grows at 36°, while *F. meliae* is near its optimum; from *F. subroseus* by lack of definite vinaceous color of mat and also different shape of chlamydospore; from *F. roseus* by faster growth rate.

24. FOMES RIMOSUS Berk. Type culture: FP-57079-S. Figures 72, 73, 120.

Growth slow, mat 2–4 cm. in 7 days, very thin, fragile, appressed velvety-cottony at center, buckthorn brown or darker to finely floccose-cottony at margin, colorless to mustard yellow, blending with color of agar; margin proper narrow to wide, colorless, very closely appressed, even; submerged hyphae variable in size 3–6(–8)μ, no clamps; superficial hyphae moderately thick-walled, colored, uniformly 3–4μ; growth at constant temperatures 1.5 cm., 21°; 3.6 cm., 26°; 4.5 cm., 31°; 1.5 cm., 36°; faint to moderately strong reaction with tannic acid medium.

In 14 days mat 4–5 cm., appressed, thin, finely floccose-cottony, buckthorn brown over inoculum becoming lighter toward margin until merging into wide colorless marginal zone; or mat thicker, floccose-cottony, with buckthorn brown or mustard yellow central zone and white, broad marginal zone; odorless.

Test tube cultures. In 4 weeks, mat either compacted, felty-cottony or loose cottony, yellow ochre to buckthorn brown, thick, with at times tendency to form shallow poroid areas.

Distinguishing characters. F. rimosus may be distinguished from other slow growing brown forms by possessing a faster growth rate at 31° than *F. robustus*, *F. Pini*, *F. Calkinsii*, *F. torulosus* and *F. igniarius*. Also the thick, compacted, crumbly mat formed in tube cultures, definitely separates it from *F. robustus*, *F. Calkinsii* and *F. torulosus* which produce

tough thin films on the agar. The mat does not peel from the agar as does *F. igniarius*, and the large size of hyphae separates it from all but *F. robustus*.

25. FOMES ROBUSTUS Karst. Type culture: 16015-S. Figures 74, 75, 121.

Growth slow, mat 2.5–3.0 cm. in 7 days, cultures showing two distinct forms, one form producing a raised, nodulose, finely felty-cottony, to woolly, *azonate* mat, antimony yellow to yellow ochre over center gradually shading to white at margin, the other form compacted, appressed, zonate, from buckthorn brown at center to yellow ochre and primuline yellow, with a wide, white, cottony marginal zone; margin proper colorless, narrow, even; submerged hyphae variable in size, 3–6(–7.5)μ, no clamps; superficial hyphae 2–4μ, thick-walled, yellowish brown rough in outline, darkening in KOH; growth at constant temperatures 2.4 cm., 21°; 3.2 cm., 26°; 3.3 cm., 31°; trace 36°; strong reaction with tannic acid medium.

In 14 days mat 5–6.5 cm., raised form evenly warm buff to buckthorn brown, color heaviest over inoculum; appressed form distinctly zoned, warm buff to buckthorn brown, not evenly colored but at times color distributed in relation to concentric zones; odorless.

Test tube cultures. In 4 weeks no distinction between forms, all isolates with raised, velvety-cottony to finely woolly fruiting pad on slant, buckthorn brown to yellow ochre; on agar cylinder homogeneous, essentially one color, buckthorn brown, tough, peeling readily from agar.

Distinguishing characters. F. robustus cannot be separated from *F. Calkinsii* with certainty in either Petri cultures or test tube cultures. In general, *F. robustus* grows at a slightly faster rate, is more vigorous in appearance and is more deeply colored. As far as test tube cultures are concerned, *F. robustus*, *F. Calkinsii*, *F. densus* and *F. torulosus* are practically identical and exhibit no features of value in identification. Temperature relations help to separate it from *F. densus*, but *F. torulosus* is more deeply colored and the submerged hyphae are smaller.

26. FOMES ROBUSTUS var. TSUGINA (Peck) Overh. Type Culture: 16836-S. Figure 122.

Growth very slow, mat 1–2 cm. in 7 days, buckthorn brown to cinnamon brown with a fine, short cottony to woolly, superficial covering, straw yellow to Naples yellow; margin thin, colorless, appressed, fairly broad, even; hyphal characters as for *F. robustus;* strong reaction with tannic acid medium.

Mat in 14 days 3–4 cm., dark brown as seen from underside of dish,

often producing a diffusion zone of considerable extent in agar, with a thin, cottony to woolly superficial covering, ochraceous buff to yellow ochre, mat proper as viewed through superficial covering dark vinaceous brown; *odorless*.

Distinguishing characters. On growth rate, color, and reaction to gallic and tannic acids. *F. robustus* var. *tsugina* falls into a group with *F. conchatus*, *F. juniperinus*, *F. igniarius*, var. *populinus* and *F. tenuis*. It may be distinguished from *F. conchatus* on general appearance since it does not produce the white, felty, cottony mat during the first 7 days of growth. *F. juniperinus* is more yellowish and homogeneous than var. *tsugina* and also produces clamps on submerged hyphae. *F. igniarius* var. *populinus* may be separated by its characteristic odor while *F. tenuis* does not form the dark colored mat proper nor the diffusion zone in the agar.

27. FOMES ROSEUS (Alb. & Schw.) Cooke. Type cultures: Snell 10 and 11. Figures 76–80, 123.

Growth slow, mat 3.5–4.5 cm. in 7 days, white to very slightly pinkish or rose-tinted, appressed, floccose-cottony, with mycelium orientated toward center, often definitely plumose, homogeneous, azonate; chlamydospores few, irregular in shape and size, with fairly thick hyaline wall, up to 40μ in length; submerged hyphae 1.5–3(–4)μ, clamps abundant; superficial hyphae similar with heavier walls and deeply staining contents, or more fibrous, non-staining up to 4μ; growth at constant temperatures 3.5 cm., 21°; 4.0 cm., 26°; 4.5 cm., 31°; 1.5 cm., 36°; no reaction with tannic acid.

In 14 days mat 7–8 cm., thin, appressed, floccose-cottony, plumose especially near margin, white over entire surface or rose-tinted over inoculum or over major portion; odor distinct, of green apples.

Test tube cultures. In 4 weeks mat thin appressed, with definite plumose appearance from pinkish buff through vinaceous cinnamon to as dark as cinnamon over inoculum, rest of mat white, or with indefinite pink or vinaceous tint, fragile.

Distinguishing characters. *F. roseus* falls in the same group as *F. pinicola*, *F. meliae* and *F. subroseus*. However, the slower rate of growth at

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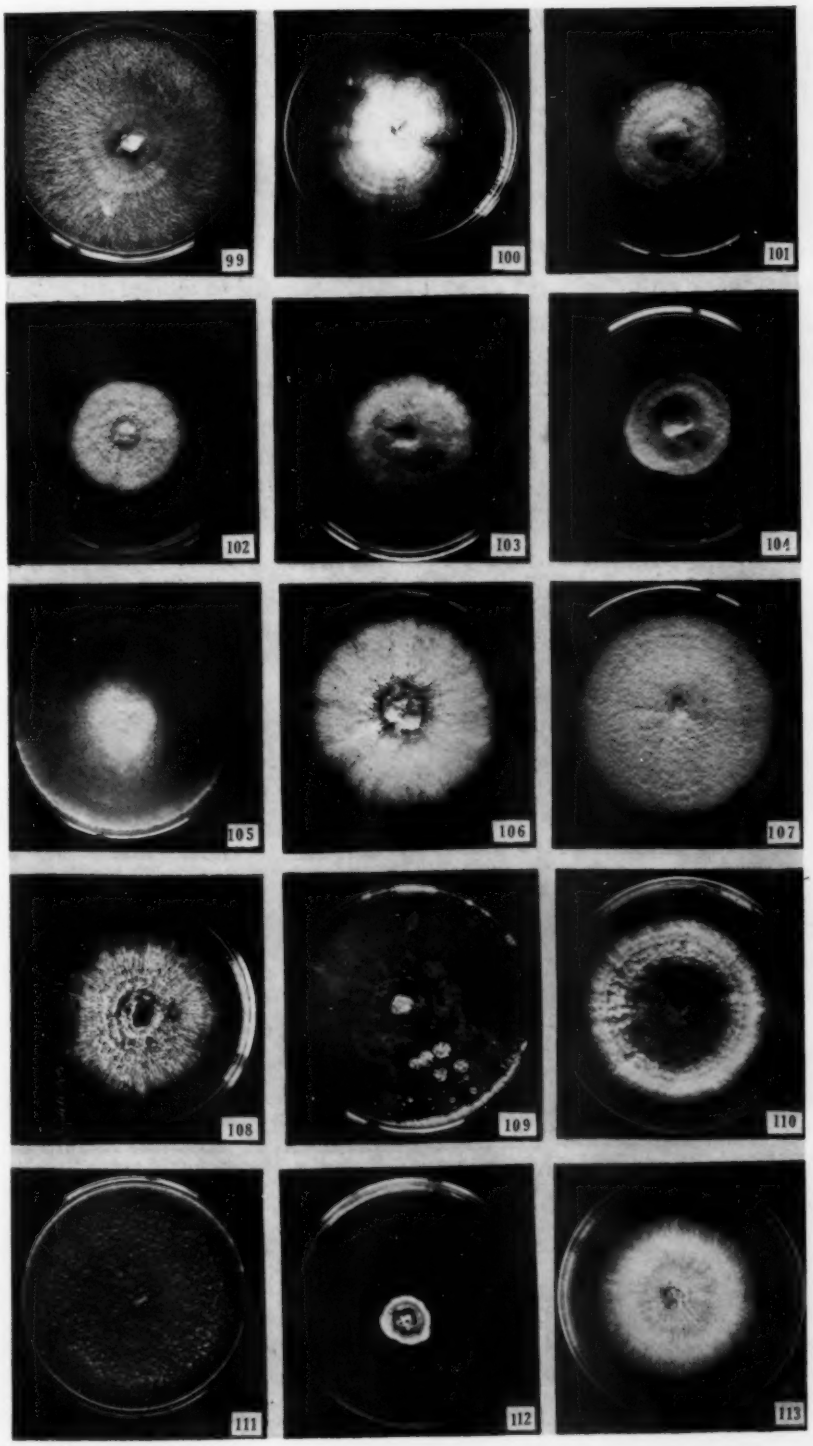
Explanation of figures 99–113

Mats on malt agar, in diffused light, at room temperature. Age 14 days unless otherwise stated.

Fig. 99. *Fomes annosus*. 100. *F. applanatus*. 101. *F. Calkinsii*. 102. *F. conchatus*. 103. *F. densus*. 104. *F. Everhartii*. 105. *F. fomentarius*. 106. *F. fraxineus*. 107. *F. fraxinophilus*. 108. *F. fulvus*, age 11 days. 109. *F. geotropus*. 110. *F. igniarius*. 111. *F. igniarius* var. *laevigatus*. 112. *F. igniarius* var. *populinus*. 113. *F. lobatus*.



Figs. 99–113. (*See opposite page for legend*)

31° and the pinkish or vinaceous tints developed in tube cultures definitely separate it from the first two, while Snell's (1928) observation as to growth rate at 31° is the only way to separate it from *F. subroseus*.

28. FOMES SCUTELLATUS (Schw.) Cooke. Type culture: 17039-S. Figures 81–84, 124.

Growth slow, mat 2.5–4 cm. in 7 days, isolates showing considerable variation, either with a prominent, appressed, central zone, cinnamon buff to brownish drab and army brown, with white appressed, granular marginal zone; or mat white, loosely felty-cottony, without any or only slight indication of a colored central zone; margin proper also variable, appressed, colorless, fimbriate to more cottony and white; submerged hyphae, 2–4(–5)μ, with clamps or with thick-walls and deeply staining contents, irregularly branched with short, knobbed sections; superficial hyphae from colored central zone, 2–4μ, thick-walled, without clamps; growth at constant temperatures 3.1 cm., 21°; 3.6 cm., 26°; 1.5 cm., 31°; 0 cm., 36°; strong reaction with tannic acid medium.

In 14 days mat 5–9 cm., size dependent upon whether mat remains white and felty without producing a central zone or whether it develops an appressed colored central zone, sayal brown, to army brown and dark greyish brown, both forms producing poroid areas on surface containing well developed basidia and spores; basidia clavate; basidiospores variable, allantoid 6–14×3.5–4μ; paraphyses-like organs found in basidial layer; no odor.

Test tube cultures. In 4 weeks mat either remaining white with wood colored or yellowish poroid areas or forming a dark, compacted region on surface of slant, separated from rest by a sharp distinct line, sometimes forming a definite pellicle, with poroid areas developing on colored portion very suggestive of pore surface of *F. scutellatus;* rest of mat white, homogeneous.

Distinguishing characters. F. scutellatus may appear in several widely differing forms which complicates its identity in culture. If it forms the typical appressed, colored central zone, it may readily be recognized, as no reactor with tannic acid approaches it in appearance; if, however, it remains white and felty, it may superficially resemble *F. fraxineus* and

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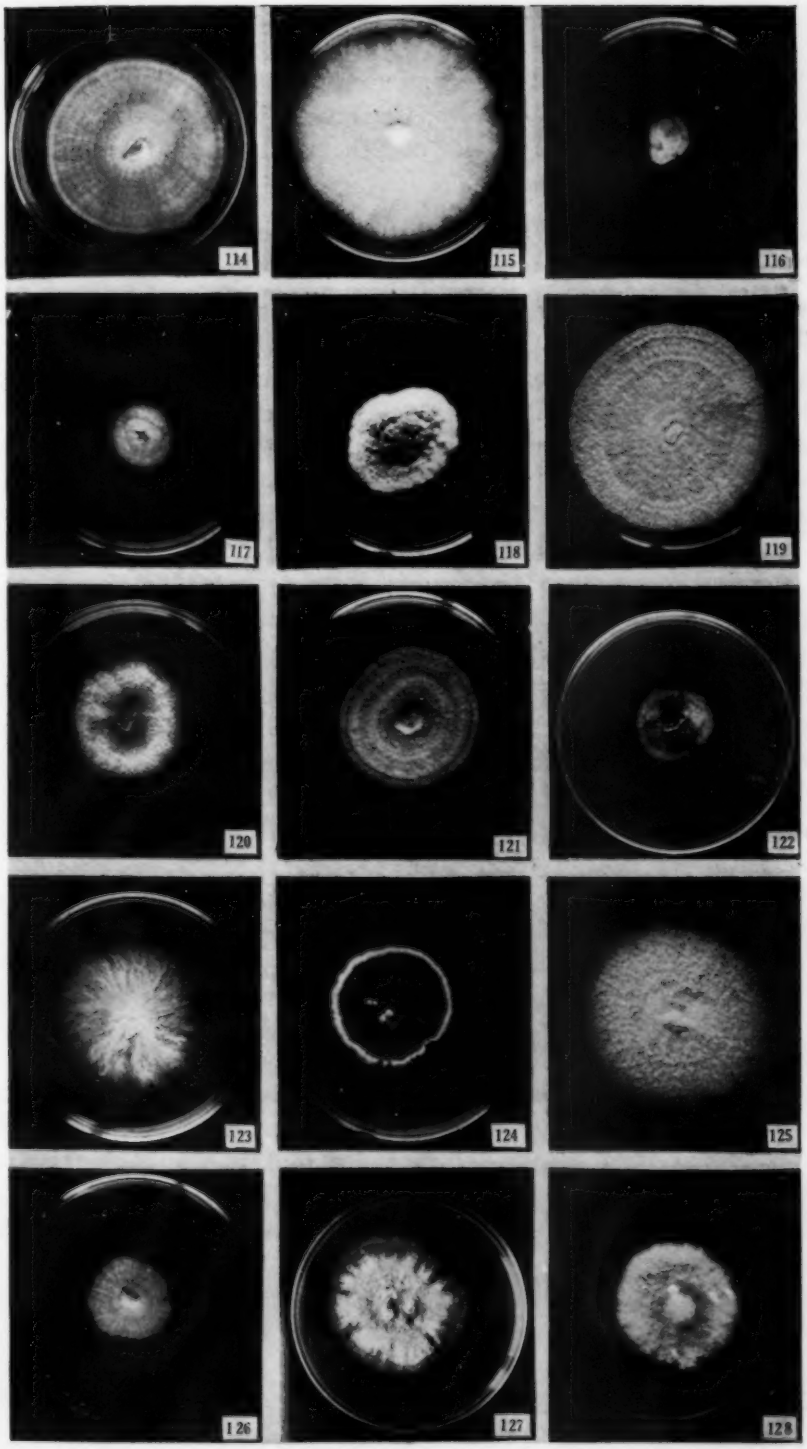
Explanation of figures 114–128

Fig. 114. *Fomes marmoratus.* 115. *F. meliae.* 116. *F. officinalis.* 117. *F. ohiensis.* 118. *F. Pini.* 119. *F. pinicola.* 120. *F. rimosus.* 121. *F. robustus.* 122. *F. robustus* var. *tsugina.* 123. *F. roseus,* age 11 days. 124. *F. scutellatus.* 125. *F. subroseus,* age 11 days. 126. *F. tenuis.* 127. *F. torulosus.* 128. *Sterile Fomes.*



Figs. 114–128. (*See opposite page for legend*)

F. fraxinophilus, but as it never produces chlamydospores may be readily separated. In test tube cultures, the production of basidia and characteristic allantoid spores coupled with the possible formation of a pellicle, makes it easy to identify.

29. FOMES SUBROSEUS (Weir) Overh. Type cultures: Snell-20, FP-57047-S. Figures 85–87, 125.

Growth fairly rapid, mat 4.5–6 cm. in 7 days, chalky white to pink or rose tinted, fairly thick nodulose-cottony to tufted floccose-cottony or felty, homogeneous, azonate, often with poroid areas; margin wide, colorless, appressed, fimbriate; chlamydospores few, lemon-shaped to elongate, 15–45×6–9μ; submerged hyphae 2–4μ, thin-walled, clamps abundant; superficial hyphae with thicker walls, deep staining contents, clamped 2–4μ; growth at constant temperatures 4.5 cm., 21°; 6.0 cm., 26°; 6.5 cm., 31°; 2.5 cm., 36°; no reaction with tannic acid medium.

In 14 days mat appressed to raised, floccose-cottony to tufted floccose-cottony, homogeneous, azonate, white at margin to irregularly light vinaceous fawn or fawn color, often with distinct poroid areas confined to inoculum or covering most of surface; chlamydospores more numerous; mature basidia rare; basidiospores short cylindric, 5–8×2–3.5μ; odor distinct, that of green apples.

Test tube cultures. In 4 weeks mat on slant either appressed, floccose-cottony, or raised, with pad on upper part of slant; pores when developed pale ochraceous buff; mat faintly pinkish with upper part light vinaceous fawn. In 8 weeks becoming light ochraceous salmon to light vinaceous fawn.

Distinguishing characters. F. subroseus may be distinguished from *F. roseus* by its much faster rate of growth at 31°.

30. FOMES TENUIS (Karst.) Type culture: 17413-S. Figures 88–91, 126.

Growth slow, mat 1.5–2.0 cm. in 7 days, closely appressed, finely floccose-cottony, indeterminate in color, greyish white to somewhat yellowish; margin proper colorless, slightly fimbriate; hyphal swellings common, resembling chlamydospores; submerged hyphae, 2–3(–4)μ, no clamps; on older portions of mat much branched and contorted; superficial hyphae up to 4μ. thick-walled, colored, very irregular, often in short lengths with many branches and swellings; growth at constant temperatures 1.5 cm., 21°; 2.0 cm., 26°; trace at 31°; strong reaction with tannic acid.

In 14 days mat 3–4 cm., white to cream or ochraceous buff and cinnamon, appressed, finely woolly to appressed downy, fragile, with at times scattered poroid areas; basidia long, clavate, 4–5.5μ in diameter, with

sterigmata up to 4μ; spores long, cylindric, slightly curved, 6–8×2–3μ; superficial hyphae often producing dark brown setae 4–6μ in diameter, in connection with poroid areas; odorless.

Test tube cultures. In 4 weeks mat appressed, compacted, yellowish to ochraceous buff, with heavy poroid areas on slant. In 8 weeks darkest colors sayal brown to cinnamon brown.

Distinguishing characters. Of the slow growing *Fomes* species producing a brown zone with tannic acid media, *F. tenuis* may be confused with *F. conchatus*, *F. ohiensis* and *F. robustus* var. *tsugina*. However, *F. ohiensis* produces abundant clamps, while *F. conchatus* is more raised, felty-cottony, and color of superficial hyphae will diffuse in KOH, while *F. robustus* var. *tsugina* is more deeply colored and has larger hyphae.

31. FOMES TORULOSUS (Pers.) Lloyd. Description based on FP-50278-R. Figures 92, 93, 127.

Growth slow, mat 2.5–4 cm. in 7 days, dense, felty-cottony to woolly, faintly to strongly zonate, color homogeneous, very slightly pale yellow at margin to yellow ochre and buckthorn brown at center; margin colorless, fairly broad, even; submerged hyphae 2–4μ, no clamps; superficial hyphae, 3–4μ, brown, thick-walled; growth at constant temperatures 2.0 cm., 21°; 3.5 cm., 26°; 3.5 cm., 31°; trace 36°; strong reaction with tannic acid medium.

In 14 days mat 5–7 cm., center dense, felty-cottony, marginal portions more appressed cottony, zonate, antimony yellow to raw sienna; odorless.

Test tube cultures. In 4 weeks mat felty cottony on agar cylinder to loose cottony on slant, often forming loose cottony pad on slant, color homogeneous, between yellow ochre and buckthorn brown, forming a tough film on agar.

Distinguishing characters. F. torulosus resembles quite closely several of the yellow to brown species of *Fomes* in its cultural characteristics. In general, *F. torulosus* is uniformly darker, buckthorn brown, while *F. robustus*, *F. Calkinsii*, *F. rimosus*, *F. densus* and *F. igniarius* are lighter in color and possess distinct marginal zones.

32. STERILE FOMES Type culture: 17352-S. Figures, 94–98, 128.

Growth slow, mat 2–3 cm. in 7 days, white, raised, loosely cottony, fragile; margin white, cottony, fimbriate; submerged hyphae 3–6μ, thin-walled, no clamps; superficial hyphae small, thick-walled, non-staining, 1.5–3μ; growth at constant temperatures 3.0 cm., 21°; 3.0 cm., 26°; 2.0 cm., 31°; trace 36°; strong reaction with tannic acid medium.

In 14 days mat 5–6 cm., white to cream buff or yellowish, raised,

loosely cottony, fragile with scattered yellowish patches, usually containing immature basidia associated with setae and slender paraphyses-like organs; surface of culture producing numerous long setae, readily visible under low-power of microscope and turning reddish when touched with KOH; basidia few to rather numerous, clavate or subglobose, up to 8μ diameter, 2- or 4-spored; spores hyaline, globose to ellipsoid, up to 8μ diameter; setae 5–8μ diameter, projecting up to 25μ in hymenial layer, on surface up to 130μ; odorless.

Test tube cultures. Mat appressed, yellowish at margin to antimony yellow where best developed, usually producing a large irregular shaped sporophore on slant; poroid areas in cross section show an agglutinated, yellowish mass, without basidia and with numerous short setae lining tubes; no functional basidia seen in abortive sporophores produced in tube cultures.

Distinguishing characters. The sterile fungus on *Betula* may be distinguished in culture by its light yellowish color, by the long setae on surface which turn reddish in KOH, and by the abortive sporophore produced in tube cultures.

KEY TO SPECIES OF FOMES CULTURED ON MALT AGAR

Mat white, often varying from faintly white to chalky white, rose colored, or at times somewhat cream colored (14 days) 1
Mat colored, yellow to brown (14 days) 15
1. Clamp connections present 2
Clamp connections absent 12
2. Forming brown zone with tannic acid medium 3
Not forming brown zone with tannic acid medium 8
3. Growth greater than 3 cm. in 7 days at 26° C in dark 4
Growth less than 3 cm. in 7 days at 26° C in dark ..... *F. ohiensis*
4. With staghorn branches on the superficial mycelium 5
Without staghorn branches on the superficial mycelium 7
5. Chlamydospores present; fruiting readily in test tube cultures, the pore surfaces vinaceous ..... *F. fraxineus*
Chlamydospores not produced; not fruiting readily; cuticular bodies present in mats 14 days old 6
6. Compacted central zone white or cream colored; marginal zone usually appressed colorless ..... *F. applanatus*
Compacted central zone yellow to avellaneous or buffy brown; marginal zone usually white, cottony, radiating ..... *F. lobatus*
7. Pores almost always produced in tube cultures; chlamydospores numerous, of two kinds ..... *F. fraxinophilus*
Pores never produced in tube cultures; chlamydospores very rare, usually absent ..... *F. fomentarius*
8. Growth 1–3 cm. in seven days at 26° C ..... *F. officinalis*
Growth greater than 3 cm. in seven days at 26° C 9
9. Forming a gelatinous layer on agar; optimum about 36° C, forming a mat 8 cm. in diameter at that temperature ..... *F. meliae*
Not forming a gelatinous layer on agar, growth inhibited or stopped at 36° C 10

10. Remaining white in cultures 14 days old; test tube cultures also white or slightly yellowish at 4 weeks ..... *F. pinicola*
Becoming rose tinted or pinkish in 14 days; mats in test tubes becoming as dark as vinaceous-cinnamon or cinnamon 11
11. Growth averaging 6.5 cm. in 7 days at 31° C. (dark) ..... *F. subroseus*
Growth averaging 4.5 cm. in 7 days at 31° C. (dark) ..... *F. roseus*
12. Producing numerous conidia on subglobose coniophores ..... *F. annosus*
Not producing conidia 13
13. Growth at 26° C. more than 6 cm. in 7 days, averaging 15 cm. ..... *F. geotropus*
Growth at 26° C. less than 6 cm. in 7 days 14
14. Mat raised, felty-cottony, at times forming rather definite dark colored mat proper; growth about 2 cm. in 7 days ..... *F. conchatus*
Mat appressed, thin, downy; hyphal swellings common; often producing basidia and spores in tube cultures; growth 2 cm. in 7 days ..... *F. tenuis*
Mat white, felted, averaging 4 cm. in 7 days; usually forming sporophore in tube cultures ..... *F. scutellatus*
15. Clamps present 16
Clamps absent 20
16. Growth slow, less than 2 cm. in 7 days at 26°; mat not producing diffusion zone in agar; color olive buff ..... *F. juniperinus*
Growth more rapid, more than 3 cm. in 7 days at 26°; mat producing diffusion zone in agar, not yellowish in color 17
17. Growth entirely inhibited at 36°; no cuticular cells formed 18
Growth slight to vigorous at 36° (1 cm. or more in 7 days); cuticular cells formed 19
18. Growth 6 cm. in diameter in 7 days at 31°; never fruiting in culture; entire surface of mat colored in tube cultures; thick-walled hyphae uniform, 1–4 μ ..... *F. fomentarius*
Growth 1.5 cm. in diameter in 7 days at 31°; usually fruiting in culture; test tube cultures either entirely white or with colored portion confined to upper part; thick-walled hyphae often with short irregular knobs and branches ..... *F. scutellatus*
19. Good growth at 36°, 4 cm. or more; oidia present; mat not divided into definite central and marginal zones ..... *F. marmoratus*
Slight growth at 36°, 1–2 cm.; only central zone colored; no spores of any kind produced ..... *F. lobatus*
20. Growth 5 cm. or greater in 7 days at 26° 21
Growth less than 5 cm. in 7 days at 26° 22
21. Mat tough, felted, two-zoned at 7 days, when old peeling readily from agar; odor of wintergreen ..... *F. igniarius* var. *laevigatus*
Mat thin, short-cottony, homogeneous, evenly colored; not peeling readily from agar ..... *F. fulvus*
22. Yellow of colored portion of mat diffusing readily in KOH 23
Yellow of colored portion of mat not diffusing in KOH 24
23. On hardwoods only; growth practically stopped at 31° ..... *F. conchatus*
On coniferous woods; growth averaging 2 cm. at 31°; hyphal swellings common ..... *F. Pini*
24. Growth slow, forming a mat 2 cm. or less in 7 days at 26°; color diffusing at times from mat into agar; mat proper dark 25
Growth more rapid than 2 cm. in 7 days at 26° or if 2 cm. not forming dark mat proper or diffusion zone in agar 26
25. Mat proper reddish-brown; superficial growth thin; usually on *Tsuga;* no odor ..... *F. robustus* var. *tsugina*
Mat proper dark; superficial growth thin to fairly dense, often with a definite border; odor pronounced sweetish; only on *Populus* ..... *F. igniarius* var. *populinus*
Mat thin, homogeneous, not forming dark mat proper; mat ochraceous buff to cinnamon; no odor ..... *F. tenuis*

26. Mat felted, usually two-zoned, center colored, marginal zone white; when 14 days old peeling from agar in a clean sheet leaving whitish film attached ..... *F. igniarius*
Mat more woolly, not peeling readily from agar, or if peeling from agar, lacking the two-zoned mat and not leaving a whitish film attached 27
27. Found only on *Robinia;* mat thin and appressed in light; test tube cultures not forming a fruiting pad nor forming a tough mat on agar ..... *F. rimosus*
Found only on *Prunus;* mat thin, essentially one color, appressed; fruiting at times readily ..... *F. fulvus*
Found on a variety of hosts; forming definite fruiting pads on malt agar in test tubes or forming sporophores 28
28. In light usually forming a dark mat proper on agar; superficial covering silky, often with a greenish tinge; mat when 14 days old turning vinaceous when rubbed with KOH; on oak. ..... *F. Everhartii*
In light usually not forming a dark mat proper, or if so, superficial covering without greenish tinge; mat woolly to woolly-cottony 29
29. Growth at 31° in dark never as great as 4 cm. in 7 days, very slight or no growth at 36° 30
Growth at 31° in dark more than 4 cm. in 7 days; fair growth, 1–2 cm., at 36°; mat in 14 days never darker than primuline yellow; superficial covering turning reddish-brown when touched with KOH ..... *F. densus*
30. Setae common on surface of culture; basidia and basidiospores often produced in scattered areas, sterile sporophore usually formed in tube cultures ..... *Sterile Fomes*
Not fruiting in culture; never producing basidia and setae 31
31. Hyphae large, up to 7.5μ; mats usually with indefinite white margin ..... *F. robustus*[3] *F. Calkinsii*
Hyphae smaller, rarely larger than 4μ; mats usually darker in color and lacking a definite white margin ..... *F. torulosus*

GENERAL DISCUSSION

Macroscopic characters. In the case of fungi in culture, three possibilities suggest themselves in making separations between groups or between individual species within a group, namely: macroscopic features, or those gross differences in color, texture of mat and the like which are readily visible to the naked eye; microscopic characters, or in reality the actual structural characters as revealed by suitable magnification and staining; and lastly the little known differences which may be classified under physiological reactions.

A brief review of the suggested macroscopic characters, as originally stated by Long and Harsch and further studied by Fritz shows that the following have been considered as being of special importance:—rapidity

[3] In general *F. robustus* and *F. Calkinsii* resemble each other too closely to be separated on basis of description alone. Certain differences, however, do exist which enable one acquainted at all with the two species in culture to distinguish them. The *F. robustus* form on *Betula* is particularly distinct but the form on oak resembles *F. Calkinsii* to the extent that one is justified in doubting that any distinction should be made between the two species. *F. robustus*, however, grows at a slightly faster rate, produces a more vigorous mat, and is more strongly colored. In cultures kept in the dark these distinctions are more apparent as *F. Calkinsii* has a tendency to remain pale and delicate while *F. robustus* becomes colored and forms a vigorous woolly mat. Even at the best, however, these differences are in degree rather than on specific points.

of growth; color of aerial and submerged mycelium; character of mat as to texture, form, etc.; staining and discolorations of the agar; and the comparative rates of growth between aerial and submerged hyphae. An attempt will now be made to evaluate these suggested criteria in light of their helpfulness in distinguishing between different species of *Fomes*.

In a general survey of any considerable number of fungi in culture probably the most apparent differences between species would be variations in color. Mycelial mats of *Fomes* species vary from colorless or pure white to all shades of yellow and brown. Usually mats that are inherently colorless or white will remain that way no matter how long they are grown, while those becoming colored will at times start as white mycelial patches later acquiring a characteristic color. Usually this color will develop in a definite period of time. This suggests the division of the species of *Fomes* into a white or colorless section and a colored section, meaning by colored some shade of yellow or brown, allowing a definite time limit, say fourteen days, for the cultures to develop. This procedure separates the species quite satisfactorily, but leaves a minimum number of species which are somewhat indefinite in regard to color and which for this reason must be included in both arms of the key.

After color, rapidity of growth, expressed in colony diameter in a definite period of time, offers most promise for identification purposes. The different species of *Fomes* show growth rates with considerable variations, which when correlated with color give valuable information as to identity. However, since room temperature measurements may show much variation constant temperature measurements are to be preferred.

Texture and type of mat produced offer valuable aids in classification of species. As little can be gained by a multiplicity of terms, Long and Harsch's original list has been followed with few additions. This list has been given under *Description of cultures*. After a little practice one becomes quite proficient in classifying the different cultures under the headings given.

Other suggested characters, such as marginal features, discolorations of the agar, distinctions between aerial and submerged hyphae and the like are too vague to be of much value as far as separation of *Fomes* species is concerned.

Microscopic characters. As reported by several workers the list of important microscopic characters includes: septations, branching, size and color of hyphae, presence or absence of clamp connections, polymorphism in spore formation and possibly number and kind of crystals produced in the medium as a result of the metabolic activities of the fungus. In a study of any group of fungi one would hardly expect that the various

microscopic characters would all be of the same importance, and it is quite possible that the value of the different characters varies from group to group. With this in mind an attempt was made to evaluate these characters in relation to the identification of the species of *Fomes* in culture.

Clamp connections with very few exceptions are either present in sufficient numbers as to be readily seen in a microscopic examination or are entirely absent. This makes entirely feasible a division of all species into those with clamps and those without clamps. Appreciable differences in type of clamp produced were not observed although some variation is apparent.

The production of secondary spores in culture adds another possible criterion for the separation of species. These secondary spores may be any one of three types. If, as in *Fomes annosus*, they are produced on definite modifications of the usual hyphal structure, they are designated as *conidia*. If they occur as pyriform or globose inflations of ordinary hyphal cells, produced terminally or intercalary, they are designated as *chlamydospores*. In one species, secondary spores of elongated form (oblong or short cylindric) have been noted. They are regarded as *oidia*. The production of spores varies somewhat with different strains of a given species and with changes in environmental factors. They may, however, be regarded as reliable criteria for purposes of identification, especially when correlated with other factors.

Other microscopic criteria of value for supplementary or correlative information include: presence or absence of staghorn branching; hyphal swellings of various kinds; setae on surface of cultures or in connection with hymenial layers, incrustations of the hyphae; presence or absence of cuticular or vesicular cells; and production of typical basidia and basidiospores.

Hyphal sizes are of little value. In general very little variation occurs between the different species, and what differences do occur are not distinct enough to be reliable. Type of branching is also an uncertain criterion as, although differences in branching do occur, these differences are difficult to describe and apt to be disturbed in the preparation of the specimens for examination. Differences in type of crystals produced are also elusive details which offer but little promise in the separation of the *Fomes* species here reported.

Response to light. The effect of the intensity of light on the form and coloration of the mats in cultures were also studied to considerable extent. In general, light has a suppressing effect on the mycelium, which is probably to be expected, and also influences the development of color. A certain amount of light is necessary for the best development of color and

cultures grown for purposes of identification are best treated when exposed to moderately strong diffused light. Growth rate varies considerably as not all species react the same to light. In the dark, the mass of mycelium produced is apt to remain relatively undifferentiated and for this reason furnish few characters of value in identification.

Temperature relations. All cultures studied were subjected to constant temperature treatment at temperatures of 21°, 26°, 31° and 36° C. These temperatures were chosen as a matter of convenience and are ones which could be most easily maintained at the time the work was carried on. The treatment of cultures during the course of constant temperature experimentation has been given under *Materials and methods.*

Each individual isolate reacts in a characteristic way as far as growth rate is concerned at a given temperature. While each isolate of a given species maintains its own constant rate of growth, the range expected for any species may be expressed within certain limits. The growth rates given for a species at a particular temperature will be the average of the different isolates.

No attempt was made to separate species into low, medium, or high temperature groups, although this can readily be done from descriptions of individual species. Most cultures of *Fomes* show a preference for temperatures between 25 and 30° C., with few able to grow vigorously at 36° C.

At higher temperatures, individual differences in strains or isolates become most apparent. Also slight variations in moisture and experimental handling produce the greatest chances for possible error.

Since intensity of light made considerable difference in colony diameter in a given time, cultures grown under constant temperature conditions, in dark, cannot be compared with cultures kept in light under same temperature conditions.

Reactions with special media. Bavendamm's procedure for detection of white rot fungi and brown rot fungi described under *Materials and methods* offers a simple means for the classification under these two heads. The brown diffusion zone produced as result of reaction with tannic and gallic acid seems constant for each species. Tannic acid is to be preferred as it turns malt agar white, against which the colored diffusion zone shows up to best advantage.

SUMMARY

1. Thirty-one species of *Fomes* were studied in detail as to their cultural characteristics.

2. The different species of *Fomes* in culture produce distinct characteristics, which makes their identification fairly certain.

3. The reaction of each species to the culture medium, if conditions remain constant, is distinct and invariable within certain limits. Variations do exist within a given species, these variations being greater in some species than in others.

4. Individual isolates of a given species retain their peculiarities for indefinite periods in culture. There appears to be no sharp change in reaction at any time and individual strains can be depended upon to remain constant.

5. There is no difference between cultures of the same individual prepared from rot or from the sporophore.

6. Macroscopic features of value in the identification of *Fomes* species in order of importance are: color of mat; rate of growth as given in terms of diameter of colony at some definite time interval; texture of mat and production of colored zones; and odor may at times be distinctive.

7. Microscopic features of value in the identification of *Fomes* species in order of importance are: presence or absence of clamp connections; production of secondary spores; existence of supplementary structures such as cuticular bodies, staghorn branches, hyphal swellings, etc.; and hyphal sizes.

8. Most *Fomes* species in culture show a decided response to light evidenced by change in texture of mat, color, and difference in rate of growth. The amount of change depends upon intensity of illumination.

9. The species of *Fomes* differ as to minimum, optimum and maximum temperatures required for growth.

10. Malt agar and potato-dextrose agar are the most suitable media for the culturing of the different species of *Fomes*.

11. Little supplementary data of use in identification can be secured by culturing the *Fomes* species on a wide variety of agars.

12. Tannic and gallic acids when added in 0.5 per cent concentration to regular malt agar furnishes a fairly positive means of distinguishing between white-rot and brown-rot fungi.

13. Cultural studies of fungi are valuable not only in the diagnosis of the cause of decay but also in ordinary taxonomic work in distinguishing between species closely related in external form. Also in distinguishing abnormal or aborted forms of the fungus.

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